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THE BIOLOGY OF THE CLINICAL PATHOLOGIST*

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The clinical pathologist is an animal of lowly origin and somewhat uncertain parentage. He had his origin as a biologic variant in response to an environmental demand. His development from a simple body with few and uncomplicated functions has been so rapid that in the life time of most of us we have seen his evolution into a complex creature with multitudinous functions and relationships. Because his evolution has been so rapid his adaptations have not been perfect and there are still evidences of maladjustment to environmental conditions. There has been the usual amount of variation in species brought about by differences in pressure of varying factors. There has been a loss here and a gain there in the constant attempt at adjustment. Some have fallen by the way side because of their inability to meet the rigorous conditions of existence. Some, who have survived, have so modified their characteristics that they closely resemble members of other families, and need to be carefully examined to determine their true character. Some have been fortunately situated and have flourished unchecked by unfavorable circumstances and stand out as examples of the finest type of development. And, then, as always, there is the occasional biologic sport which appears without known cause and refuses to be classified by any known rules into any known group.

Biologically, the clinical pathologist still shows many primitive characters and, in some respects, resembles the lower forms of animal life. Conversely, in his evolution, he has lost some of the capacities which were prominent in earlier days. For example,

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while the ability to do work has increased, the ability to maintain normal nutrition has not kept pace and, in a medium weakened by competitive organisms of lower grade, he finds it difficult to maintain himself.

A comparison with one form of simple organism, with which you are all familiar, namely, the bacterium, will serve to illustrate the retention of some fundamental primitive characters.

Bacteria may be roughly divided into parasitic and non-parasitic organisms. So may clinical pathologists. Some of them maintain an independent existence by virtue of necessity; others, by choice. Some live at the expense of others with little or no effort to contribute to the mutual welfare, while others bend every effort to justify their existence. Some maintain themselves with reluctance, others with enthusiasm. Altered conditions may bring about a change in this regard in both organisms and pathologists and some who begin as pure parasites may adapt themselves to an independent status. Some refuse modification to meet changed conditions and survive or perish depending upon their own vigor and the severity of the environmental pressure.

Again, bacteria may be divided into the pathogens and non-pathogens. Occasionally, we see a situation that reminds us that this division may be made in us also. There are some individuals who always are making trouble for some one, usually their host. Existence apart from trouble-making is, for them, unthinkable. Others, fortunately enough, are never concerned in making trouble but carry on at peace with the world. As with bacteria, the non-pathogens are greatly in the majority and carry on a large number of activities that are vital for the normal life of the community. Some lowly organisms, for example, are able to fix atmospheric nitrogen; a thing which man accomplishes only by expenditure of vast sums of money and the building of elaborate machinery. These humble servants have kept us alive by their labors though they have been almost unknown, unhonored and unsung. Their efforts have been accepted without compensation and little thought of gratitude. They have been taken for granted and passed by. To draw an analogy would be painful.

Some bacteria are able to live on a very simple diet. Almost

any kind of food will do. They are not fastidious but tenacious of life under difficult conditions. Circumstances that would mean the death of the less hardy, mean nothing to them. Wherever they happen to fall, there they develop and only actual starvation, or summary destruction, stops them. And then there are those who do not, or think they do not, have their opportunity in one location and move about from place to place; never satisfied, never happy, always looking for something better and more nearly what they think they deserve. Everything has to be exactly right for them to maintain themselves long in any location. A great deal of pampering and petting, and most careful handling is necessary to keep them growing.

There are organisms whose chief claim to fame is that they are gas producers. The gas itself is usually of no value save as a means of identifying the organism. Sometimes it is the kind of gas, more often the amount, and frequently the circumstances leading to its formation that characterize the species. Further discussion on this point would be valueless.

Then there are the luminiferous bacteria. In some mysterious way they bring light out of darkness. They are not mere reflectors but makers of light and shine out "like a good deed in a naughty world."

And then, sad to relate, there are some whose life is accompanied by alcoholic fermentation.

If the analogy may be continued further it may be said that bacteria, which are quite definitely of pure strain ancestry, may be seen to develop in smooth or rough colonies, and the smoothness and roughness are more than superficial characteristics. Why pursue this throught further?

In recent years we have learned that bacteria may be completely destroyed by an ultra-microscopic substance, the bacteriophage, probably originated by the bacterium itself. The clinical pathologist finds himself similiarly attacked, and sometimes destroyed by the technician for whose beginning the pathologist himself was responsible.

Finally, it may be remembered that from the time of our first knowledge of bacteria until the present, there has been no change

in the salient characteristics of the bacteria known to us. May the same permanence of type be prophesied for clinical pathologists? For the family the answer is, probably, in the affirmative; for certain individual species, I think, there is no hope. The clinical pathologist who came into being because of a demand from his professional confréres for a peculiar kind of service and who established himself as an independent unit and continues to maintain himself without hospital or teaching connections is rapidly becoming extinct. Through competitive forces of various kinds he finds conditions more and more unfavorable and is maintaining himself at present only in comparatively few strategic locations.

This, to my mind, is a most interesting illustration of the evolution of a species, the radical change in the conditions affecting it, with the inevitable result following inexorable law. Much blame for the difficulty has been laid at the door of the technician whereas we forget that economic law operates in the practice of medicine as elsewhere. It is economically unsound for a high-priced worker to spend his time doing something that a low-waged worker can do as well. A manufactured article, to be sold, must be marketed at a reasonable price which must yield a reasonable profit to the producer. To keep the price within reasonable bounds and insure his profit the manufacturer cannot pay skilled men for unskilled labor. Labor costs must be commensurate with the labor done. Some of us, I fear, have forgotten that economic principles have their play in medicine as in business and have expected, through some more or less miraculous intervention, to have them set aside for our benefit. We should not be surprised when such intervention does not make its appearance. It is still true that "the laborer is worthy of his hire" but in these days when there is so much talk about the cost of medical care, uneconomic factors must inevitably succumb. The internist, or the surgeon, who finds himself confronted with the necessity of doing certain work for a fee which the patient may be able to pay and who requires assistance from the clinical pathologist and roentgenologist may find that after these are paid, what he has left is totally inadequate compensation for his own labor. To

meet this condition he resorts to some means by which he may secure what he terms routine examinations at a lower price and the clinical pathologist grumbles because of the loss of patronage. These conditions are not satisfactory to the clinical pathologist, or to the clinician. In due process of time evolutionary adjustment will bring about a greatly improved method of caring for this work. How much some of us may suffer, and how many of us shall be ground between the upper and the nether millstone depends upon whether we may be able to adapt ourselves to the rapidly changing conditions. That we, ourselves, may appreciably control the conditions seems to me a futile hope. After all, these are evolutionary processes moving in conformance to law. That some of us are hurt in the change is unfortunate but does not stop the movement.

It is probably not far from the truth that our specialty in medicine has come about as a result of evolutionary forces; that these forces are still operating; that changes in conditions necessitate changes in us; that some of us may survive while others perish. It is important, I believe, to recognize the fact that these various conditions are controlled by law, and that while we may be able to modify, or adjust, certain factors, to refuse to recognize these laws, to fail to admit their controlling influence, means a futile hope, impossible of realization.

On the other hand an intelligent adaptation offers the prospect of a greater usefulness and the greater security which we so much desire. The situation is not hopeless. A knowledge of the forces at work, a recognition of the laws by which they are governed, and a vision of the future based on this knowledge make it possible to adapt ourselves to the changing demands. An intelligent adaptation should see us through these days of transition and, though we may emerge vastly changed, we shall remain an integral and important factor in the science of medicine.

Program for the
1931 Convention at Philadelphia

The programs at the annual conventions are each year becoming longer and in order to prevent their becoming too long or too crowded it will be necessary to make some selection from the titles submitted.

Those who contemplate reading a paper at the coming convention are requested to send *as soon as possible* the title and an abstract of the paper to the Chairman of the Program Committee, Robert A. Kilduffe, M.D., Atlantic City Hospital, Atlantic City, New Jersey.

HEMATOLOGICAL ASPECTS OF AGRANULOCYTOSIS AND OTHER DISEASES ACCOMPANIED BY EXTREME LEUKOPENIA*

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The primary object of this communication is to present a classification based on the blood picture and histological changes in the bone marrow of the various diseases which are associated with a marked leukopenia.

The symptoms and blood picture are fairly diagnostic in certain conditions as in pernicious anemia, nutritional anemia or sprue, the various forms of splenomegaly (Gaucher's disease, splenic anemia and hemolytic icterus), and certain types of diseases of the lymphnodes after biopsy (Hodgkin's disease and lymphosarcoma). In Hodgkin's disease and sprue, septic manifestations and necrotic ulcerations of the mucous membranes are occasionally associated with the leukopenia.

Well-defined infectious diseases such as typhoid fever, and occasionally influenza, meningococcic sepsis, subacute endocarditis (with splenomegaly) and miliary tuberculosis may be accompanied by a marked diminution in the number of leukocytes. In a miscellaneous group (table 6) a certain number of cases of hepatitis (catarrhal jaundice), hyperthyroidism and a few cases of infectious mononucleosis (generalized transitory lymphadenopathy), especially in women, were noted to have a leukopenia; these patients often had fever and prostration, but no ulcerations of the mucous membranes were present. In a few women and one man there was persistent leukopenia of unexplained origin, or so-called idiopathic leukopenia.

* Read before the Ninth Annual Convention of the American Society of Clinical Pathologists, Detroit, Michigan, June 20–23, 1930.

The main groups which will be discussed pertain to the cases with a profound leukopenia (100 to 4,000 white blood cells), often showing septic manifestations, with necrotic lesions in the mucous membranes.   Agranulocytosis is the most important member of this group.   This, according to Schultz,[27] is a clinical entity of an apparently rapidly fatal septic condition with a characteristic leukopenia, associated with marked diminution or disappearance of the polymorphonuclear neutrophils or granulocytes.   The differentiation of this disease from conditions simulating it is important.   These are aplastic anemia (aleukia hemorrhagica), acute purpura hemorrhagica with a marked leukopenia and leuko-penic leukemia.   The underlying etiologic factors in this large group are not known.   Certain toxic factors, however, may pro-duce similar leukopenic and aplastic syndromes as in radium, x-ray, or benzol poisoning and following intravenous administra-tion of salvarsan.

Diphtheria, septic sore throat, Vincent's angina and infectious mononucleosis may resemble agranulocytosis or agranulocytic angina[9] symptomatically but the blood pictures do not show the characteristic profound leukopenia in such cases.   Vincent's spirilli may occasionally be present in the necrotic lesions of agranulocytosis.   The marked septic manifestations of agranulo-. cytosis are absent in the other various forms of ulcerative pharyn-gitis.[28]

I have had the opportunity of studying ninety cases of various diseases associated with marked leukopenia, exclusive of perni-cious anemia and various forms of splenomegaly, malignant lymphadenopathies and cases treated with x-rays.   According to the clinical manifestations and course, the following classification can be presented:

    I. FATAL AGRANULOCYTOSIS (table 1)
        A. Without anemia (Schultz), 4 cases
        B. With anemia (Brown), 6 cases
        C. Malignant leukopenia, 2 cases
    II. BENIGN AGRANULOCYTOSIS—Recovered cases, 14 cases (table 2)
    III. APLASTIC MYELOID DISORDERS (table 3)
        A. Acute aplastic anemia (aleukia hemorrhagica), 9 cases

B. Chronic aplastic anemia, 10 cases

C. Panmyelophthisis, 3 cases

D. Purpura hemorrhagica (with leukopenia), 1 case

IV. LEUKOPENIC LEUKEMIA, 15 cases (table 4)

V. TOXIC LEUKOPENIA OR APLASTIC MYELOID DISORDERS, AS A RESULT OF POISONING WITH BENZOL, RADIUM, AND X-RAY, ARSENIC ARSPHENAMINE, 6 cases (table 5)

VI. MISCELLANEOUS CASES (NUTRITIONAL ANEMIA, CHLOROTIC ANEMIA, ENDOCRINE DISORDERS, HEPATITIS, OBSCURE INFECTIONS, IDIOPATHIC LEUKOPENIAS), 20 cases (table 6)

FATAL AGRANULOCYTOSIS (TABLE 1)

A. Without anemia

These cases presented the typical syndrome first described by Schultz,[28] namely, fever, necrotic manifestations of the mucous membranes, prostration and occasionally jaundice. Türk reported a similar case in 1907, complicated by an endocarditis. In 127 cases, previously reported, women have been affected in 88 per cent. I have observed four additional cases, all in middle-aged women. The blood changes are typical and identical with those described by Schultz,[28] the white cells ranging from 200 to 4,000. There was almost complete disappearance of the polymorphonuclear neutrophils with relative increase in the percentage of lymphocytes. Occasionally a few plasma cells and macrophages were present. The bone marrow, examined in cases 1 and 2, showed the characteristic lesions such as complete disappearance of the myeloid cells. Only red cells, lymphocytes, plasma cells, megakaryocytes, and occasionally macrophages were seen.

B. With anemia

The first case of this type was reported in this country by Brown, as "a fatal case of acute primary infectious pharyngitis with extreme leukopenia." Schultz[29] did not accept such cases at first as agranulocytosis, but later modified his views. The anemia is usually moderate and may exist prior to the onset, or may result from the infection. In case 7, the patient suffered from menorrhagia, which caused the anemia. Thirty-four sim-

TABLE 1
Fatal Agranulocytosis

CASE	SEX	AGE	LOCATION OF NECROSIS	BLOOD CULTURE	COURSE	HEMOGLOBIN per cent	ERYTHROCYTES	LEUCOCYTES	PLATELETS	POLYMORPHONUCLEARS per cent	LYMPHOCYTES per cent	MONOCYTES per cent	PLASMA CELLS per cent	MACROPHAGES per cent	BONE MARROW
1	F.	33	Pharynx; anus; skin	Strep. [hemolytic] and Esch. coli	Relapsing	78	4,704,000	600	610,000		95	5			Typical
2	F.	48	Nasopharynx	Staph. aureus [hemolytic]	Acute	84	4,350,000	400	240,000		99		1		Typical
3	F.	60	Pharynx	Strep. [hemolytic]	Acute	85	4,400,000	500	310,000		85	3	12		Not examined
4	F.	49	Pharynx; anus; skin	Strep. [hemolytic]	Relapsing	85	4,550,000	300	320,000	1	65	12	5	17	Not examined
5	F.	47	Tonsils; rectum; skin	Ps. aeruginosa and Strep. [hemolytic]	Acute	65	3,000,000	2,000	Normal		88	4	8		Typical
6	F.	50	Tonsils	Not done	Acute	70	3,810,000	1,700	210,000		89	11	3		Not examined
7	F.	54	Tonsil	Not done	Acute	62	3,000,000	400	200,000	2	89	6	5		Not examined
8	F.	28	Pharynx	Strep. [viridans]	Acute	74	4,100,000	1,200	250,000*	14	88	3		5	Typical
9	M.	50	Fauces; skin	Sterile	Acute	60	4,000,000	950	200,000*		83	3	5		Not examined
10	M.	62	None	Sterile	Acute	40	2,500,000	400	140,000	22	70	4	4		Not examined

* Followed by terminal thrombocytopenia.

ilar cases, twenty-nine in women and five in men have been reported. I have observed six additional cases (four women and two men). The hemoglobin varied from 60 to 74 per cent, red blood cells from 3,000,000 to 4,100,000, and white cells from 400 to 2,000. The blood platelets were normal in three cases but in cases 8 to 10 a terminal thrombocytopenia developed. The polymorphonuclear neutrophils varied from none to 14 per cent. The symptoms and course were similar to those in the group without anemia. The bone marrow in cases 5 and 8 was typical. In case 5 the bone marrow also showed numerous macrophages.

C. Malignant leukopenia

Cases 11 and 12 are interesting from the standpoint of the differential blood picture which showed a normal number of neutrophilic polynuclear cells, so that the diagnosis could not be agranulocytic but rather hypoleukocytic or malignant leukopenia. The symptoms, however, in case 11 were the same as in the agranulocytic type, fever, intense prostration and widespread ulceration of the pharynx and tongue. A few plasma cells and macrophages were occasionally present in addition to the premature polymorphonuclear cells.

In case 12 the symptoms, however, were those of an acute intense infection, such as high fever, up to 107°F., extreme prostration, and slight cough, but no ulcerations of the mucous membranes could be found. Some obscure lung signs were present in the chest indicating a bronchopneumonia, and the abdomen felt rather doughy. There was a secondary anemia and marked leukopenia (1,800 to 3,200 white blood cells). The x-ray examination of the chest later showed evidence of miliary tuberculosis. The differential blood counts on cases 11 and 12 are in table 1a.

In such cases there is an overwhelming sepsis involving the bone marrow. This blood picture is certainly the one expected to result from such a primary infection. Such cases may prove to be of great importance in indicating the pathogenesis of agranulocytosis in which the infection is probably secondary and the changes in the bone marrow are possibly primary.

TABLE 1a

	CASE 11	CASE 12
Hemoglobin	74 per cent	60 per cent
Erythrocytes	3,800,000	3,200,000
Leukocytes	900	2,700
Platelets	430,000	180,000
Polymorphonuclears, young	12 per cent	2 per cent
Polymorphonuclears, staff	53 per cent	24 per cent
Polymorphonuclears, segmented	2 per cent	28 per cent
Lymphocytes	26 per cent	42 per cent
Monocytes	4 per cent	3 per cent
Myelocytes (neutrophilic)	2 per cent	None
Myeloblasts	1 per cent	None
Macrophages	None	1 per cent

AGRANULOCYTOSIS WITH RECOVERY (TABLE 2)

These are cases of agranulocytosis without a history of anti-syphilitic treatment, or without other known cause. Twenty-seven patients (twenty women and seven men) have been reported as recovered following an attack of agranulocytosis. The variations in the leukocytes in these cases ranged from ninety-nine to 3,000. The largest number of recoveries, six cases, were reported by Friedemann. I have under observation at the present time fourteen cases which have recovered. Most of these cases had a profound leukopenia, varying from 800 to 1,500, and a few cases with a leukopenia varying from 3,000 to 4,600. All of the cases presented the agranulocytic syndrome of fever, more or less prostration and ulcerations of the mucous membrane. Case 24 showed no pharyngeal ulcerations, but signs of a bronchopneumonia were present. In most of the cases the blood picture did not correspond to the fatal type. In a few cases anemia was present; this was chlorotic in type (see table 6). The average leukocyte count was also higher as only one case had less than 1,000 leukocytes. The polynuclear cells varied from 5 to 35 per cent, and lymphocytes were relatively increased. Case 22 is

TABLE 2

AGRANULOCYTOSIS—RECOVERED CASES

CASE	SEX	AGE	LOCATION OF NECROSIS	BLOOD CULTURE	COURSE	HEMOGLOBIN per cent	ERYTHROCYTES	LEUCOCYTES	PLATELITS	POLYMORPHONUCLEARS per cent	LYMPHOCYTES per cent	MONOCYTES per cent	PLASMA CELLS per cent	MACROPHAGES per cent	BONE MARROW
13	F.	21	Tonsils; gums	Sterile	Acute	80	4,500,000	1,600	190,000	16	79	3	1	5	Not examined
14	F.	48	Tonsils	Sterile	Acute	72	3,525,000	4,600	220,000	21	61	7	4		Not examined
15	F.	45	Tonsils; skin	Sterile	Acute	80	4,420,000	4,400	360,000	5	35	58	2		Not examined
16	M.	45	Gums	Not done	Acute	90	4,300,000	3,600	220,000	21	61	0			Not examined
17	F.	50	Tonsil	Not done	Acute	39	3,000,000	1,500	320,000	9	88	3	1		Not examined
18	F.	32	Tonsil	Not done	Acute	95	5,100,000	5,100*	350,000	35	58	3			Not examined
19	F.	38	Tonsils	Not done	Acute	90	4,400,000	3,000	240,000	30	60	4			Not examined
20	F.	56	Tonsils	Not done	Acute	85	4,600,000	1,800	180,000	18	56	15			Not examined
21	F.	49	Tonsil; rectum	Not done	Acute	60	3,500,000	800–1,800	370,000	48	42	10			Myeloid hyperplasia
22	F.	45	Tonsils; sinusitis	Sterile	Chronic	32	4,100,000	3,100	200,000	88†	10	2			Not examined
23	M.	45	Tongue	Sterile	Chronic	52	3,136,000	1,400	210,000	12	50	16†			Not examined
24	F.	50	None; broncho-pneumonia	Sterile	Acute	60	3,450,000	2,240	200,000	29	54	17			Normal; light hyperplasia
25	F.	17	Tonsil	Not done	Acute	85	4,800,000	4,500	250,000	24	72	4			Not examined
26	F.	36	Tonsils	Not done	Acute	80	4,500,000	2,400	320,000	22	60	16	2		Not examined

* Two weeks after the attack.

† Aleukocytosis polynucleosis.

‡ Seventeen per cent myelocytes and myeloblasts.   Leukopenia persists five years later.

of the leukopenic type, with 88 per cent polymorphonuclear cells. A certain number of the cases in this group are still being followed and cases 22 and 23 after five years without symptoms still show a leukopenia as low as 1,500. In following up such cases I am led to believe that there is an associated constitutional factor which is either transitory or permanent in the production of the leukopenia and which may account for the peculiar reaction to the infection.

Case 22, an unusual case of unexplained edema, shows some evidence of endocrine disturbance such as myxedema, and sparcity of hairs, and a lowering of the basal metabolism. Biopsy of the sternum was done in this case and the bone marrow was found to be hyperplastic. At the time the bone marrow was removed, there was a marked leukopenia—2,000 white blood cells in the peripheral blood.

In case 23 nephrectomy was performed for calculus pyonephrosis. The blood was examined after the operation on account of sluggish progress of the wound and hemorrhage. The patient complained of a sore tongue on which a few ulcerations were present. The blood picture showed a marked leukopenia, a few myelocytes and myeloblasts and marked diminution of the polymorphonuclear cells. Following a blood transfusion, the patient made an uneventful recovery. The myelocytes and myeloblasts were not found on subsequent examinations, but for the past five years the blood examinations showed a constant leukopenia, neutropenia and relative lymphocytosis. Otherwise the blood picture was normal. The general condition is good and the patient is symptomatically well.

This large group of recovered cases, rather heterogeneous in character, corresponded to the fatal group of cases of agranulocytosis as far as the early symptoms were concerned. There is no doubt, however, that in the fatal cases the underlying pathological lesions in the bone marrow were relatively different. Here there was a complete absence of myeloid elements. In cases twenty-two and twenty-four which recovered the bone marrow showed evidence of hyperplasia. The septic manifestations in this latter group were most likely primary, with some inhibitive or toxic re-

action on the bone marrow. In some cases there was an underlying constitutional disturbance[3], resulting in diminution of the leukocytes, prior to the onset of infection, while in others the leukopenia was the result of the infection. In the fatal agranulocytic group there seemed to be a widespread disease of the bone marrow, of an aplastic nature, limited to the myeloblastic and myelocytic portion of the bone marrow, so that there was a lack of resistance against even slight infections on account of the absence of the polymorphonuclear cells in the peripheral blood. In a few cases there may be temporary improvement or remission, but the patient usually succumbs during the second or third relapse.[26] According to the blood picture and the granulopoietic aplasia in the bone marrow, fatal agranulocytosis appears to be a clinical entity.

APLASTIC MYELOID DISORDERS (TABLE 3)

Acute aplastic anemia, described by Ehrlich, usually runs a rapid course. Severe anemia is present from the onset and is out of proportion to the hemorrhages as the result of the associated hemorrhagic condition. The blood picture is typical and shows a constant and progressive reduction in the number of red cells. The white cells and blood platelets are also greatly diminished. The coagulation time is usually normal, and the bleeding time is increased. The tourniquet test is positive and the clot retraction is absent or diminished. These reactions are similar to those of purpura hemorrhagica. The course of aplastic anemia varies from one week to three months and may be prolonged by blood transfusions. In the chronic form of this disease the course may run as long as five years. The hemorrhagic manifestations are not so severe as in the acute type. The mode of exitus in cases of aplastic anemia is usually exsanguination or cerebral hemorrhage.

In a few cases secondary infections may occur with necrotizing lesions of the mucous membranes and skin, and clinically the condition may simulate agranulocytosis. This particular septic course may be present from the onset. The clinical syndrome showing severe anemia, hemorrhagic manifestations and necrotizing lesions in the mucous membranes has been called "aleukia

TABLE 3

CASE	AGE	SEX	LOCATION OF NECROSIS	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	PLATELETS	POLYMORPHONUCLEARS	LYMPHOCYTES	MONOCYTES	OUTCOME
				per cent				per cent	per cent	per cent	
Acute aplastic anemia											
27	22	F.	Intestines	29	1,500,000	800	20,000	25	60	5	Dead
28	24	M.	Subcutaneous	31	1,600,000	800	5,000	14	85		Dead
29	24	M.	None	28	1,300,000	900	10,000	8	82	8	Dead
30	17	M.	Pharynx	22	1,330,000	800	2,000	12	86	2	Dead
31	20	F.	Pharynx	15	828,000	1,200	1,500	19	67	5	Dead
32	21	F.	None	22	1,800,000	8,800	10,000	14	81	5	Dead
33	20	M.	Pharynx	27	1,100,000	1,000	80,000	22	72	1	Dead
34	50	M.	Pharynx	35	1,820,000	1,200	10,000	16	82	2	Dead
35	10	F.	Pharynx	18	690,000	750	4,000	1	96		Dead
Chronic aplastic anemia											
36	6	M.	None	25	1,600,000	1,200	4,000	3	94		Dead
37	7	M.	None	68	3,500,000	2,400	2,200	22	69	8	Dead
38	6	M.	None	44	2,048,000	2,100	30,000	3	97		Dead
39	7	F.	None	28	1,600,000	4,000					Dead
40	3	F.	Pharynx	45	2,800,000	2,400	70,000	27	65	10	Dead
41	10	M.	None	69	3,900,000	3,800	20,000	22	72	5	Dead
42	16	F.	Pharynx	30	1,460,000	1,900	40,000	22	75	3	Dead
43	9	F.	None	26	1,600,000	2,900	25,000	35	60	4	Dead
44	62	F.	Pharynx	62	3,200,000	600*	40,000	14	66		
45	25	F.	None	35	1,500,000	3,000	60,000	40	51	8	Dead
Chronic myelophthisis											
46	4	F.	Pharynx; skin; periostium	32	2,240,000	3,800	290,000	26	73	1	Dead
47	17	F.	Broncho-pneumonia	18	1,190,000	3,800	310,000	24	68	7	Dead
48	8	M.	None	32	1,700,000	3,200	130,000	38	58	2	Unimproved

* Sixteen per cent plasma cells.

hemorrhagica," by Frank. Later investigations may show that this septic type may possibly be a distinct clinical entity, closely

related to aplastic anemia. Aleukia hemorrhagica may resemble purpura hemorrhagica associated with a leukopenia and without splenomegaly. In purpura hemorrhagica, however, an anemia is not present at first but develops as the hemorrhages increase. The blood examinations in such a case in a male aged eighteen years, are given in table 3a.

TABLE 3a

	FEBRUARY 13, 1930	FEBRUARY 18, 1930	FEBRUARY 19, 1930
Hemoglobin	105 per cent	73 per cent	62 per cent
Erythrocytes	6,000,000	4,000,000	3,900,000
Leukocytes	2,600	2,800	2,800
Platelets	20,000	20,000	50,000
Polymorphonuclears, staff	20 per cent	24 per cent	5 per cent
Polymorphonuclears, segmented	46 per cent	36 per cent	48 per cent
Polymorphonuclears, eosophilic	1 per cent	None	4 per cent
Lymphocytes	32 per cent	35 per cent	36 per cent
Monocytes	11 per cent	5 per cent	7 per cent
Coagulation time	12 minutes	9 minutes	
Bleeding time	4 minutes	92 minutes	
Tourniquet test	Positive	Positive	
Clot retraction	None	None	

The examination of the bone marrow in this particular case also showed a very marked diminution of the granulocytes. Similar cases of this type which I have observed, did not show any evidence of a septic invasion.

Some cases of thrombocytopenic purpura hemorrhagica with leukopenia may be accompanied by a splenomegaly. These cases are more benign and usually recover following splenectomy.[24]

There is still another type of an aplasia of the bone marrow which is known as "panmyelophthisis." I believe that this term should be restricted to cases which are characterized by a severe anemia and leukopenia, but in which there is no hemorrhagic tendency. The blood platelets are normal at first but may diminish late in the disease. In a few such cases sepsis with or

without necrotic ulcerations may occur. These cases are usually chronic and afebrile however.

Twenty-one cases of the various forms of aplastic anemia have been observed in the past fifteen years. At the onset the blood examinations always showed a severe anemia varying from 10 to 50 per cent. The leukocyte count in most of the cases was below 3,000 although rarely a transitory rise took place. There was a relative lymphocytosis, but the polynuclear cells varied from 5 to 40 per cent. In late stages of the disease, with an associated infection, the polynuclear cells sometimes showed a profound depression. In all of the cases of acute and chronic aplastic anemia there was a thrombocytopenia (blood platelets varying from 2,000 to 20,000), and as a rule this was associated with a marked hemorrhagic diathesis. The differential count showed an occasional myelocyte or myeloblast, rarely over 5 per cent; reticulocytes were absent or reduced.

The bone marrow was examined in these three groups and was apparently the same in all cases. Usually there is a hypoplasia of the red cells. The megakaryocytes were very greatly diminished and sometimes were absent. The leukocytic elements usually consisted of a moderate number of lymphocytes, myelocytes and myeloblasts. In the myelophthisic group there was considerable lymphocytic infiltration and usually more megakaryocytes were seen. A diminution of the mature myeloid cells was evident in all types of aplastic anemia.

LEUKOPENIC LEUKEMIA (TABLE 4)

This group very frequently causes confusion between aplastic anemia and other cases associated with secondary infections, especially of the tonsils, followed by necrotic manifestations simulating agranulocytosis. The blood picture in this group varies with respect to the red blood cells and the blood platelets which are usually diminished. The white blood cells may be very greatly reduced in number and associated with a constant increase of abnormal myeloid cells. Leukopenic leukemia may not necessarily be the same disease entity as myeloid leukemia of the leukocytic type, but may be the result of sclerotic or fatty

changes in the hematopoietic organs with myeloid metaplasia of the liver, spleen and lymph-nodes, with the presence of premature myeloid cells in the circulating blood. In other cases the typical lesions of leukemia are found in the bone marrow.

TABLE 4

LEUKOPENIC LEUKEMIA

CASE	AGE	SEX	LOCATION OF NECROSIS	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	PLATELETS	POLYMORPHONUCLEARS	LYMPHOCYTES	MONOCYTES	MACROPHAGES	MYELOBLASTS	OUTCOME
				per cent				per cent	per cent	per cent	per cent	per cent	
49	12	M.	Pharynx	39	1,920,000	2,200	205,000	17	51	2		27	Dead
50	7	M.	Pharynx	40	2,300,000	4,800	260,000	5	55	4	6	29	Dead
51	34	M.	None	56	3,800,000	3,700	60,000	1	12	1		69	Dead
52	42	F.	Pharynx	50	2,600,000	2,000	6,000	6	52	2	5	35	Dead
53	46	F.	None	23	1,400,000	2,500	140,000	61	26	3	4.4	3.6	Dead
54	56	F.	None	30	1,400,000	4,000	20,000	41	39	8	4	5	Dead
55	45	M.	None	33	1,600,000	2,000	20,000	34	48	3	8	8	Dead
56	7	M.	None	29	1,800,000	1,500	10,000	6	60		1	31	Dead
57	53	F.	Pharynx	62	3,240,000	4,000	20,000	5	37		3	51	Dead
58	63	M.	None	56	3,200,000	3,100	220,000	13	36			49	Dead
59	50	F.	None	32	1,700,000	2,000	50,000	12	56			32	Dead
60	44	F.	None	65	3,500,000	2,500	80,000	24	40	2	2	28	Dead
61	6	M.	None	30	1,000,000	750	5,000	30	20	2		48	Dead
62		F.	None	40	2,800,000	2,200	100,000	4	64	2	6	6	Unimproved
63		F.	None	16	800,000	4,000	70,000	33	27	2	18	18	Unimproved

The differential count in these cases is of great importance and should be studied very carefully with the Jenner-Giemsa stain; myeloblastic cells may also be detected by means of the oxidase reaction. In certain cases of profound leukopenia the method of Mengler may be used in concentrating the white blood cells, by

centrifuging citrated blood and using the top layer of the red blood cells for making the blood smears. In this manner a larger number of leukocytes are presented in the blood smears in cases of marked leukopenia. Occasionally myeloblasts have been found in agranulocytosis, as in the case of von Domarus. Case 23 showed a moderate number of myeloblasts on the first examination. The patient recovered but for the past five years has shown a constant leukopenia (less than 2,000) and relative lymphocytosis, but no premature myeloid cells. However, the constant appearance of myelocytes and myeloblasts in the peripheral blood is a characteristic diagnostic observation. The leukocytes may greatly diminish suddenly and fall to as low as 750 (case 61). Herz reported a typical leukopenic leukemia with 800 leukocytes, mainly myeloblasts in type. Frank made note of a similar case, which was followed by apparent recovery and later developed a leukocytic myeloblastic leukemia. A few cases reported as agranulocytosis are undoubtedly leukopenic leukemia with or without secondary necrotic septic manifestations. Table 4 shows the reports of the blood pictures in fifteen cases of leukopenic myeloid leukemia. Leukopenic lymphoblastic leukemia is very rare, especially cases with less than 5,000 leukocytes.

TOXIC LEUKOPENIAS AS A RESULT OF POISONING WITH BENZOL, RADIUM AND X-RAY, ARSENIC AND ARSPHENAMINE

About forty cases with profound leukopenia associated with or following anti-syphilitic treatment with neosalvarsan have been reported. The symptoms in some cases resemble those of agranulocytosis and in others those of aplastic anemia. Aubertin and Lévy have suggested the following classification of the agranulocytic types:

1. Agranulocytic form
2. Associated forms
   A. Agranulocytosis, moderate anemia and hemorrhagic diathesis
   B. Agranulocytosis, severe anemia and hemorrhagic diathesis
3. Formes frustes—same as above but only slight changes of the polymorphonuclear cells

Five additional cases have been observed. Case 64 apparently resulted in a chronic aplastic anemia. So far no ulcerations of the mucous membranes have appeared.

Cases 65, 66, and 67 belong to the agranulocytic group. Case 66 made a complete recovery. The bone marrow from the sternum was examined during the leukopenic period of this patient and was found to be hypoplastic. The bone marrow in case 67 showed the typical findings of agranulocytosis, with a complete disappearance of myeloid cells and apparent replacement by lymphocytes and plasma cells.

TABLE 5

AGRANULOCYTOSIS FOLLOWING SALVARSAN THERAPY (CASES 64 TO 68) AND BENZOL POISONING (CASE 69)

CARD	AGE	SEX	LOCATION OF NECROSIS	HEMOGLOBIN per cent	ERYTHROCYTES	LEUKOCYTES	PLATELETS	POLYMORPHONUCLEARS per cent	LYMPHOCYTES per cent	MONOCYTES per cent	PLASMA CELLS per cent	OUTCOME
64	36	M.	None	45	3,200,000	2,400	30,000	37	60	3		Improved
65	38	F.	Pharynx	42	2,250,000	500	50,000		94	4	2	Dead
66	35	F.	Pharynx	67	3,650,000	3,200	150,000	5	31	54		Well
67	53	M.	Pharynx	78	3,800,000	1,000	60,000		86		14	Dead
68	35	F.	Pharynx	73		2,300		45	41	14		Dead
69	45	M.	None	27	1,800,000	1,700	30,000	34	52	7		Improved

In all cases there was a secondary anemia, more or less severe, a leukopenia (white blood cells 2400) and a marked thrombocytopenia (30,000 to 60,000 platelets). There was marked diminution of the polymorphonuclear neutrophils and a relative leukocytosis. In case 67, 14 per cent plasma cells were present.

One case of benzol poisoning is at present under observation. The blood picture corresponds to that of aplastic anemia. The condition is gradually improving after transfusions. The red cells and blood platelets have increased, but the leukocytes are still less than 2000 in number.

The leukopenia following x-ray and radium treatment for non-leukemic conditions is sometimes followed by a profound leukopenia which subsides after the treatment has been stopped. A few cases of x-ray poisoning have been reported in which the findings are those of chronic aplastic anemia (Wegelin).

In Hodgkin's disease, the x-ray treatment may produce a marked leukopenia, sometimes associated with a marked reduction in leukocytes. There are also cases of Hodgkin's disease which may have a constant leukopenia, as a result of bone marrow involvement, or as a result of aplasia of the bone marrow from x-ray treatment. The leukocytes in some cases may fall as low as 240 and the symptoms before exitus may resemble those of agranulocytosis (Miller).

Martland has called attention to the anemia and leukopenia occurring in radium workers which he calls leukopenic anemia of the regenerative type. I have made examinations of three cases of radium poisoning and can confirm the blood picture of aplastic anemia as described by Martland. In one case the blood picture was similar to that of aplastic anemia. In two others there was a severe anemia and leukopenia, but the blood platelets were not greatly diminished as in cases of panmyelophthisis and the relative percentage of the lymphocytes was undisturbed. In these two cases the typical osteonecrosis of the mandible was present. The staff polynuclear cells were increased in these two patients. In one case, basophilic-like granules (toxic) were present in the staff polynuclear cells.

MISCELLANEOUS CASES (NUTRITIONAL ANEMIA, CHLOROTIC ANEMIA, ENDOCRINE DISORDERS, HEPATITIS, OBSCURE INFECTIONS, IDIOPATHIC LEUKOPENIAS)

This series of cases indicates the relative frequency of leukopenia in certain conditions without the necrotic lesions in the mucous membrane. The leukocytes in this particular group varied from 3,000 to 6,000. Most of the cases had a relative lymphocytosis. Six of these cases ran a typical course of infectious mononucleosis, fever, generalized lymphadenopathy, and splenic enlargement. A few cases of this type may develop

necrotic lesions in the mucous membranes and may resemble agranulocytosis. Such patients recover. Case 15 was of this type and showed an unusual increased number of monocytes resembling the monocytic angina of Schultz.[30] The cases of von Domarus and Kohn, as a result of salvarsan, were similar in character. Some cases of chlorotic anemia and liver disease also showed a leukopenia. A few cases of typical influenza were also associated with a leukopenia. In a few cases the cause of the leukopenia could not be determined, and were considered constitutional in character.

In one case of cirrhosis of the liver, with a white cell count of 4,000 and 74 per cent polynuclear cells, the patient later developed a gangrenous appendicitis. The leukocyte count during this attack rose to 8,000 with about 78 per cent polynuclear cells, of which 12 per cent were staff forms. In similar cases where a leukopenia precedes the infection, a leukocytosis may be considered to range from 7,000 to 10,000, in contradistinction to the leukocytosis of 14,000 to 20,000 white cells in the normal individual.

The leukopenia in these various miscellaneous cases indicates the difference in the reaction of certain individuals, especially women, and that leukopenia is much more prevalent than is generally suspected.

ADRENALIN REACTION IN CASES OF AGRANULOCYTOSIS

The reaction of adrenalin in normal individuals is well known. From fifteen to forty-five minutes after the injection of 1.0 mgm. of adrenalin subcutaneously, one usually observes a leukocytosis of a varying degree. Licht and Hartmann observed no leukocytosis in their case of agranulocytosis following the injection of adrenalin. Benhamou found a definite increase of the leukocytes in a fatal case, and Stockinger found a definite leukocytosis in a benign case of agranulocytosis following the injection of adrenalin. In leukopenic leukemia and aplastic anemia a marked rise of the leukocytes is found after adrenalin injection. Adrenalin produced only a slight increase in the leukocytes from 2,000 to 3,000 in case 4 a fatal case of agranulocytosis. The rhythm of the white

cells was also studied in this same case, but there was only a very slight inconstant change every fifteen minutes in the number of the leukocytes. The adrenalin reaction may prove to be an important indication of the potency of the bone marrow and other tissues and differentiate the genuine cases of agranulocytosis from allied conditions.

THEORETICAL CONSIDERATIONS OF LEUKOPENIA

Leukopenia may be considered the result of intrinsic or extrinsic influences on the hematopoietic organs which are the source of the leukocytes, namely: the bone marrow; lymph-nodes, and possibly other areas, such as the reticulo-endothelial tissue which may have leukopoietic potentialities. The extrinsic factors in the regulation of the white blood cells are the vegetative nervous system, infection, and the acid-base equilibrium of the body.

According to the recent observations of Mueller and Hoff, there is some proof that the vegetative nervous system influences the circulating leukocytes. The action of various drugs such as adrenalin, atropin and pilocarpin, ingestion of milk, as in the Widal, hemoclastic crisis, are well known examples of reactions on the vegetative nervous system. Mueller has shown that the intradermal injection of foreign protein, such as aolan, produces a peripheral leukopenia. He has also pointed out that the red blood cells are not involved in this mechanism. His experiments also indicate that there is a splanchno-peripheral correlation, so that when the leukopenia occurs in the peripheral blood vessels, there is a marked increase of the white blood cells in the splanchnic area. The injection of adrenalin produces a reversal of the leukocytic distribution (Mueller and Hoelscher[22, 23]).

Infection is another important influence on the leukopoietic organs. This is possibly the most outstanding factor in the study of leukopenia. The ordinary infections produce a marked polynucleosis, especially in the early stages, such as the staff polynuclear cells, which, according to Schilling[25] are accompanied by marked regenerative changes in the bone marrow. According to this observer, there are three phases in the reaction of the hematopoietic organs to infection: (1) The polynuclear

TABLE 6

MISCELLANEOUS CASES ASSOCIATED WITH LEUKOPENIAS

CASE	DIAGNOSIS	AGE	SEX	HEMOGLOBIN per cent	ERYTHROCYTES	LEUKOCYTES	PLATELETS	POLYMORPHONUCLEARS per cent	LYMPHOCYTES per cent	MONOCYTES per cent	PLASMA CELLS per cent	OUTCOME
70	Infectious mononucleosis	28	F.	70	4,650,000	4,700	220,000	34	53	7	5	Well
71	Infectious mononucleosis	8	F.	82	5,050,000	4,500	180,000	30	65	3		Well
72	Infectious mononucleosis	36	M.	85	4,500,000	4,300	320,000	50	43	4		Well
73	Infectious mononucleosis	22	M.	100	5,400,000	4,600	260,000	36	55	8		Well
74	Infectious mononucleosis	26	F.	68	3,840,000	4,800	320,000	26	68		2	Well
75	Tibial periostitis; arteriosclerosis	70	M.	85	4,800,000	3,500	320,000	10	80	3	3	Unimproved
76	Hepatitis	40	M.	78	4,000,000	3,700	200,000	55	38	4		Well
77	Hepatitis; jaundice	35	F.	84	5,100,000	4,200	200,000	27	44	42		Well
78	Neurosis	42	F.	82	4,000,000	3,800	350,000	50	47	3		Unchanged
79	Infectious mononucleosis	32	F.	92	4,900,000	3,200	320,000	59	34	6		Well
80	Carcinoma and Hodgkin's disease	56	M.	47	2,500,000	900*	110,000	61	24	12		Dead
81	Hepatitis	29	M.			4,200		44	40	6		Well
82	Cirrhosis of liver	65	F.	42	2,500,000	3,200	310,000	75	18	5		Dead
83	Cirrhosis of liver	50	M.	100	5,340,000	4,000	190,000	74	20	6		Well
84	Ecchymoses	38	F.	76	4,950,000	4,000	240,000	57	39	3		Improved
85	Menorrhagia		F.	70	3,800,000	2,300	140,000	36	41	12		Well
86	Chlorotic anemia	22	F.	56	5,000,000	4,200	420,000	39	50	11		Well
87	Nutritional anemia	45	M.	36	2,000,000	3,800	50,000	62	25	12		Well
88	Hyperthyroidism	40	F.	65	3,800,000	3,000	260,000	40	55	5		Improved
89	Influenza	40	F.	87	5,800,000	4,200	450,000	61	38	1		Well

* Received x-ray treatment.

increase; (2) monocytic increase; and, (3) increase of lymphocytes and eosinophils during the post-infectious stage.

Other types of atypical infections are accompanied by an increase of the lymphocytes or monocytes (infectious mononucleosis). The relation of certain types of organism associated with such lymphocytosis has been suggested. Witts and Welb found a monocytosis following injections of the Bacterium monocytoides in rabbits. Not infrequently infectious mononucleosis may be preceded by a moderate leukopenia and may not show a leukocytosis at any stage of the disease (cases 70–74, 79, table 6).

Certain forms of long-continued streptococcus viridans bacteremias may be associated with an increase of the reticuloendothelial cells or macrophages in the ear blood (Schilling[25] and others). Such cases may show a marked leukopenia in the finger blood and leukocytosis in the ear blood. However, sub-acute bacterial endocarditis with splenomegaly, may be accompanied by marked leukopenia. Certain types of fulminating sepsis (meningococcus, staphylococcus, typhoid fever, miliary tuberculosis (case 12)) involve the bone marrow to a very great extent, and may be associated with a marked leukopenia. Other types of sepsis of long duration, bacteriemia and even overwhelming fulminating bacteriemia are accompanied by leukocytosis. In such instances the young and staff polynuclears are increased from 20 to 60 per cent. Large numbers of these young polynuclear cells show basophilic granulation. These granules have been called toxic granules by Gloor and Barta believes that these granules are possibly ingested particles. These cells also indicate a poor prognosis. Vacuolization of the polynuclear cells with toxic granules is also sometimes present in these severe infections. In the agranulocytic conditions the few polynuclear cells occasionally found may show the toxic granules.

Hormones and the acid-base equilibrium of the blood[12] may also be factors in the regulation of the number of circulating leukocytes. It is well known that certain products broken down in the stomach by the action of the gastric juice, may have a profound influence on the red blood cells[c] but, as yet, sufficient proof has

not been brought forward concerning the action of similar substances on the leukopoietic tissue. In the miscellaneous group, cases of hyperthyroidism and hypothyroidism are noted, with marked leukopenia. One of the cases of agranulocytosis which recovered also showed evidence of hypothyroidism associated with severe chlorotic anemia. Friedemann pointed out a possible relationship of endocrine disturbances with leukopenia in one of his reports on agranulocytosis.

The constitutional factor may be of very great importance, and may explain the increased frequency of agranulocytosis in females. It is not infrequent for some of the patients who develop agranulocytosis to have a constant leukopenia or a tendency to a leukopenia as a result of some primary disturbance of the granulopoietic tissues. These tissues may be somewhat diminished, compared to the normal, or may be sluggish in the production of polynuclear cells in the presence of an infection. The bone marrow may become rapidly exhausted from even a slight infection, so that the defense mechanism against infections may be reduced to a minimum, resulting in the characteristic lesions which are found in agranulocytosis, namely: the necrosis without an inflammatory reaction. This occurs in areas, where numerous organisms are usually present, such as the respiratory and gastro-intestinal tracts. Kleeberg showed that in animals rendered agranulocytic, the anti-bacterial power in the blood is considerably diminished.

Leukopenia may be either of a transitory nature or permanent. In one of my recovered cases of agranulocytosis, the leukocyte count has never been above 2,000 for the past five years. In other cases, leukopenia has been found prior to the onset of the disease. The study of the underlying cause of the constitutional factor is likely to help in solving the problem. The leukopenia in itself, which may be found in certain women who have never had real attacks of agranulocytosis, may not be associated with any symptoms, which appear when the secondary infection takes place. In the eighty-nine cases reported in this paper, 64 per cent were in females. In the agranulocytic groups there is a marked preponderance, 85 per cent of females affected.

DISCUSSION OF BLOOD PICTURES IN LEUKOPENIC STATES

In the various groups outlined, the blood picture may be considered of diagnostic and prognostic importance. In the fatal cases of agranulocytosis, the main characteristic is a profound leukopenia of less than 1,000 white blood cells and the complete disappearance of the polynuclear leukocytes (granulocytes), whereas the blood platelets are normal except for a terminal thrombocytopenia. In the rare form of malignant leukopenia, the symptoms may be identical, but the profound leukopenia is accompanied by a corresponding diminution of the three types of leukocytes. The polynuclear cells in this condition are mainly of the staff variety with toxic granules. A moderately severe anemia may be present.

The recovered cases of agranulocytosis exceptionally show such a profound leukopenia, and are rarely accompanied by complete disappearance of the polynuclear cells. As in the fatal cases, the red cells are usually normal or slightly subnormal and there is an absence of a hemorrhagic tendency.

In the aplastic disorders of the bone marrow which involve more than one system, there are three well-defined groups, although some variations occur within each group. In aplastic anemia, either acute or chronic, a marked diminution of all the cellular elements of the blood are found, mainly anemia, leukopenia, and thrombocytopenia, and the differential count shows a relative lymphocytosis. In addition to the hemorrhagic diatheses, septic manifestations may occur (aleukia hemorrhagica), with a profound terminal leukopenia and disappearance of the polynuclear cells. In panmyelophthisis there is a deficiency of the hematopoietic and leukopoietic elements, without involving the blood platelets. Terminal sepsis simulating agranulocytosis can also occur in this aplastic type of blood disease. An atypical type of purpura hemorrhagica with marked leukopenia, but without splenomegaly may occur. In this condition the leukocytes and blood platelets alone are involved, and anemia results from loss of blood.

All these aplastic bone marrow changes, including agranulocytosis, may result from poisoning with benzol, radium and x-ray, arsenic, and arsphenamine.

Leukopenic leukemia is sometimes accompanied by a profound leukopenia, but the differential blood count is characterized by the presence of premature myeloid cells, such as myeloblasts and myelocytes and rarely lymphoblasts. Secondary septic manifestations may occur and occasionally simulate agranulocytosis.

There is sufficient evidence to indicate that the histological examination of the bone marrow also shows characteristic lesions in these various groups. These usually correspond to the blood changes, but in some cases there is marked discord between the leukopenia and the hyperplastic histological picture of the bone marrow especially in the benign cases of agranulocytosis. In this latter group, the cause of the leukopenia must be considered extrinsic in character. In the main groups, however, such as fatal agranulocytosis, the aplastic anemias and leukopenic leukemias, the bone marrow changes correspond with the blood picture. This points to a primary disease of the bone marrow. Septic manifestations result from the deficient formation of the granulocytes and hemorrhagic disorders are due to blood platelet deficiency.

SUMMARY

1. Ninety cases of marked leukopenia that resulted from various disturbances are presented.

2. The main cause of the leukopenia is believed to be primary in aplastic constitutional disturbances of the bone marrow:

A. Fatal agranulocytosis as the result of some profound disturbance in the formation of the granulocytic element.

B. Aplastic anemia as the result of widespread aplasia of all the elements in the bone marrow.

C. Panmyelophthisis resulting in diminution of the red and white blood cells.

D. Purpura hemorrhagica with leukopenia where the white blood cells and blood platelets are involved.

3. Leukopenic myeloid leukemia may symptomically resemble some of these blood conditions, but the constant presence of the characteristic myeloblasts and myelocytes or lymphoblasts in the peripheral blood is important for the differentiation of this particular disease.

4. A benign form of agranulocytosis also occurs as a result of infection. The bone marrow in such cases is not aplastic.

5. Direct toxic action on the bone marrow caused by benzol, radium, x-rays, salvarsan, may produce various blood pictures of agranulocytosis and other aplastic conditions (aplastic anemia, myelophthisis, and purpura hemorrhagica).

6. A large number of miscellaneous cases are also noted. These show a more or less severe leukopenia (infections, liver disease, obscure leukopenias, Hodgkin's disease, chlorotic anemia, pernicious anemia, and carcinoma with metastases to the bone marrow, and various forms of splenomegaly).

7. The study of the blood picture establishes certain criteria for *the differentiation of the various diseases associated with marked* leukopenia.

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AGRANULOCYTIC SYNDROMES*

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Ever since the classical studies of Arneth, Tuerk, von Jagic and others, the pathological reactions of the leukopoetic system have been observed and followed with intense interest by a large number of clinical hematologists who contributed valuable data to the clinical branch of hemopathology.

The great variety in the leukocytic picture found under various circumstances as well as under apparently identical situations led to discrepancies of opinions and to divergences in interpretation of the observations on the peripheral blood. We need only to point out the interesting question of the malignant myeloblastic reaction of the blood, of the monocytic blood picture, of the rapid disappearance of granulocytes from the blood stream, and so forth, all hematological reactions, complexes and syndromes not uncommonly observed in connection with systemic infections and toxemias, often caused by one and the same bacterial group.

In 1922, W. Schultz tried to separate a peculiar nosological entity under the name of agranulocytosis. These cases were characterized by sudden onset with high fever and malaise, development of ulcerous-necrotic, diphtheritic-gangrenous processes in the mouth, particularly localized on the tonsils, pharynx, gingiva, and soft palate, or in mucous surfaces of some other organs, such as the esophagus, colon, and vagina, and absence of icterus and hemorrhagic diathesis. Enlargement of the spleen and liver was only occasionally observed whereas lymph nodes did not show any swelling. The blood changes were characterized by a rapid drop in the number of white blood cells (leukopenia) with a nearly complete disappearance of granulocytes (neutro-

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philes and eosinophiles).   The erythrocytic blood picture did not exhibit any alterations and the platelets were also unaffected. A lethal outcome followed rapidly.   In two of the first five cases Schultz cultured pneumococci from the blood stream.   Schultz was emphatic in postulating for this sharply outlined symptom complex the term agranulocytosis, which he desired to introduce as a definite disease.

Schultz' agranulocytosis is not the result of a specific etiological factor (*Diplococcus pneumoniae*, Streptococcus [hemolytic], *Escherichia coli*, *Pseudomonas aeruginosa* were found), but is, as the author claims, a characteristic symptom complex deserving to be accepted as a definite nosological entity.

Since 1922, many observations have been made and papers have been published in rapid succession, partly confirming, partly modifying and supplementing the original statements of Schultz. Some authors included in their discussion particularly the damages to and reactions of the erythropoetic system, cases which according to our experience show a complete functional exhaustion of the entire bone marrow.

SUMMARY OF CASES

Since 1925, we have observed twenty-two cases of abnormal reactions of the leukopoetic system in individuals showing ulcerous-gangrenous processes of the oral cavity.   This present study, which is presented as a preliminary report, is based upon seventeen cases, involving twelve males, ranging in age between five and fifty-two years, and five female patients, whose ages varied between twenty and eighty-three years. All but two of the patients died.

Our cases can be summarized as follows: so-called agranulocytic angina, eleven cases; so-called monocytic angina, one case; aleukia, two cases; symptomatic agranulocytic angina in septic endocarditis, two cases, and symptomatic agranulocytic angina in septic (streptococcic) anemia, one case.

Of particular interest were the necrotic ulcerative affections of various mucous surfaces, so commonly observed in connection with agranulocytic syndromes.   The gangrenous processes of the

mucous membranes were localized mainly in the mouth and involved its various structures, such as the gums, tonsils, soft palate, lips, and buccal mucous surface, and in one case the nose. This condition raises the question as to whether the ulcerous gangrenous processes were primary or developed as secondary manifestations of the agranulocytic complex.

It would seem that the onset in some cases is a simple tonsillar angina (streptococcic?) or a more or less severe streptococcic sore throat (rhinopharyngolaryngitis) which is followed by various degrees of bone marrow reactions with subsequent gangrenous processes of the mucous surfaces. Sometimes this gangrenous process is of a secondary nature resulting from hemorrhagic infiltrations and infarction of the nasal and oral mucous membranes.

This condition may be likened to the neutropenic hypoleukocytic blood picture in some instances of acute phlegmonous gangrenous appendicitis incited by hemolytic streptococci.

A sore throat antedated the onset by a few days or weeks in seven instances, while sinus (ear) trouble was found mentioned four times by the patients as having preceded their present severe illness for a short time. Sudden onset was definitely established in three cases.

In the eleven instances of agranulocytic angina, icterus was observed twice and hemorrhagic purpura four times.

As to the bacteriological investigations, in seven cases streptococci were observed, twice in smears from the local ulcerous-gangrenous areas of the mouth. In one case, streptococci were obtained from the local lesions and from the urine. In five cases, in which autopsies were performed, a hemolytic streptococcus was cultured from the splenic pulp.

In eleven instances the behavior of the blood platelets was investigated and a thrombocytopenia of more or less marked degree was detected in eight cases. In two instances of the so-called agranulocytic angina, the blood platelet count was within the lower boundary of normal thrombocytic values. One case of recrudescent septic endocarditis, appearing clinically under the picture of an aleukia, was characterized by a complete absence of the blood platelets (athrombocytosis).

Postmortem examinations were performed in seven instances, and in each a toxic or septic picture was disclosed. The reaction of the spleen was found to depend upon the predominence of the toxic or septic symptoms; in the latter instance enlargement of the spleen was present. In cases observed at autopsy, a splenic tumor, partly of septic character, was disclosed five times.

DISCUSSION

It is interesting to note that abundant hemorrhages from the necrotic ulcerous tissues, particularly from the uterus and vagina, nose, and other mucous surfaces will aggravate an already existing anemia.

The thrombocytopenia and athrombocytosis, if accompanied by quantitative and qualitative changes in the leucocytic values, are significant manifestations of an intense septic damage to the bone marrow.

In some agranulocytic cases, the qualitative analysis of the peripheral blood will disclose an increase in monocytic blood elements, occasionally presenting the picture of a pronounced mononucleosis. The monocytic reaction is a peculiar, pathological bone marrow reaction, which results from a disturbed leukopoesis due to infectious or toxic influences upon, or irritations of, the bone marrow. The so-called monocytic angina (Schultz) belongs to this group.

We had opportunity to observe accurately and study four cases of benzol poisoning, characterized from the beginning by a sharp drop in the leukocytic counts accompanied by a considerable decrease in the number of polymorphonuclears. It clearly appeared that the leukopenic and granulocytopenic values were running parallel, namely, the more pronounced the decrease in the number of the white blood cells, the more scarce were the granulocytes. Hyperchromatic anemia of regenerative type and thrombocytopenia completed the hematological data. We did not observe in these instances any septic temperatures nor gangrenous conditions of the accessible mucous surfaces, at the beginning. One of these cases showed, however, as a terminal event, severe hemorrhages from the nose, mouth, and vagina, and.

shortly before death, development of an ulcerous stomatitis and gangrenous mastitis with septic temperature and profound anemia, a clinical picture very similar to and suggestive of an aleukia.

The agranulocytic leukopenia, which is characteristic of the blood of individuals suffering from benzol poisoning, appears to be the result of direct damage to the leukopoetic system (central deleterious action: osteomyelotoxic), while leukocytolytic processes (peripheral destruction) seem to occur also. In view of simultaneous injurious effects upon the erythropoetic system, it is plausible to conclude that the benzolic agranulocytosis is mainly a primary bone marrow damage due to osteomyelotropic properties of benzol. This dangerous poison first inhibits and then paralyzes the bone marrow functions.

The various pathological reactions of the leukopoetic and erythropoetic systems, encountered in instances of elective action of damaging agencies of toxic and infectious nature upon bone marrow, as found in the morphological blood picture, are of great symptomatic value and have been summarized as follows, omitting completely the behavior and reactions of the lymphatic apparatus:

1. Neutrophilic (hyper-) leukocytosis
2. Neutropenic white blood picture (normal leukocytic values)
3. Neutropenic hypoleukocytic reaction
4. Agranulocytosis with leukopenia
5. Myeloblastic leukemic reactions

The agranulocytosis may be accompanied by toxic aplastic anemia, toxic hemolytic anemia, or by a normal erythrocytic blood picture. The thrombocytes may show normal values or they may be markedly decreased (thrombocytopenia) or completely absent (athrombocytosis).

CONCLUSIONS

Our cases are characterized by a toxic elective action upon the leukopoetic system with destruction of granulocytes and concomitant abnormal reactions of the bone marrow, processes which

manifest their presence by quantitative and qualitative changes of the peripheral blood.

The elective action upon the bone marrow tissues, the osteo-myelotropic action, explains also the often observed damages to other functional parts of the bone marrow, to the erythropoetic system and to the thrombogenesis, with resulting anemia and thrombocytopenia. We are confronted thereby with a complete destruction, anatomical and functional, of the blood forming tissues, as a result of deleterious influences of some toxic and infectious agencies.

The hematological picture is initiated by transitory functional disturbances of the bone marrow, followed by progressively developing damages and increasing destruction of its tissue, with subsequent total, fatal elimination of its entire function, involving all its functional units and vital activities (leukopoesis, erythropoesis, thrombocytopoesis).

From the morphological behavior of the peripheral blood, we can quite positively conclude in a given case the character and extent of the pathological-physiological disturbances and alterations of the affected bone marrow.

The variations in reactions are pathological functional expressions of individual character, of a constitutionally weak, functionally readily insufficient and easily vulnerable bone marrow, depending also upon the type and virulence of the noxious agent.

The constitutional (functional) inferiority of the bone marrow is vividly expressed in those instances in which an increased functional demand is urgently and vitally needed, but the bone marrow tissues respond with an alarming collapse and a rapid, complete exhaustion.

STUDIES ON SCHILLING COUNT IN APPENDICITIS*

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A review of recent literature shows a striking lack of confidence by surgeons in the blood count as an aid in the diagnosis of appendicitis. It is interesting to note that men like Deaver and Woodall discuss this subject without mentioning the leukocyte count; Lincoln claims it to be an unreliable sign in either diagnosis or prognosis; Reese states, "to these symptoms may be added leucocytosis, which, however, is totally unreliable"; Bolling derives no information therefrom; and neither Wallace, Koritschoner, Hinton or Jackson are impressed by the count as a dependable sign, even questioning its value in diagnosis. Many authors, among whom are Smith and Brooks, report normal blood counts in cases with severely infected appendices.

In many instances, only the total white cell count is considered; others consider the relationship of the total cell count to the percentage of polymorphonuclears, some using various charts or indices of resistance to illustrate this relationship more graphically. This lack of accord in interpreting the leukocyte count led Hellwig in 1928 to conclude that, "Fowler's opinion that it is difficult to estimate the exact diagnostic value of the leucocytosis is at the present time as true as it was in 1900." From such a study of the literature, one inevitably carries away the impression of a striking lack of understanding by the surgeon of the present day concept of the physiology and pathology of the blood as determined by the total and differential cell counts.

A review of the development of the blood count and of the theories of its interpretation may help to explain the reason for this chaos. The first enumeration of the cellular elements of

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the blood was made, in 1851, by K. Vierordt.[10]   Erlich's introduction, in 1879, of staining methods for the study of blood smears
made possible the separation of leukocytes into classified types,
and initiated a new epoch in the study of hematology.   Einhorn[10]
is credited with the first differential cell count from a smear, in
1884, but his interest was in the technic of the count rather than
its interpretation.   In 1905, Sondern concluded from a study of
the results of a large number of blood counts rather than from
theoretical reasoning, that, "the increase in the relative number
of polynuclear cells is an indication of the severity of toxic
absorption and the degree of leucocytosis is an evidence of the
body resistance toward infection."   These observations were
immediately confirmed by Gibson, who evolved a chart showing
this relationship graphically.   Wilson modified Gibson's chart by
the addition of a line of resistance.   In 1919 Walker verified the
work of Sondern, Gibson and Wilson and presented a mathematical formula, the index of resistance, which was, "a ready and
tangible method of determining the degree of resistance and
which expresses essentially the same thing as Gibson's chart, but
is a more accessible means of interpreting a blood picture than
the standard chart."   These writers noted exceptions in children
to whom Gibson's resistance chart did not apply because of the
variations in the normal leukocyte count from that of the adult,
and by Sondern, "in conditions in which pus is confined so that
no toxic absorption occurs or when the purulent exudate is of
tuberculous or typhoid origin."   More recently, Menninger and
Heim, and Kilduffe, in 1924, and Jones and Crocher, and Neal
and Robnett, in 1927 concur in a general way in the above
conclusions.

    In 1904, about the time Sondern was carrying on his observations, Arneth attempted to gauge the severity of infections by
the study of blood smears.   His discovery that variations in the
nuclear structure of polymorphonuclear cells corresponded with
the severity of the infection was epochal and far reaching.   The
observed variations in nuclear structure he grouped into classes
according as they contained "one or more nuclei," meaning
nuclear lobes.   By this method of classification, the cells fell into

five groups which were further subdivided according to nuclear conformation, so that in all twenty distinct neutrophilic forms were listed.   His work awakened a more than transitory interest both abroad and in this country, but the method was so cumbersome and time-consuming that its practical application was discouraged, in spite of the fundamental deductions possible from its use.

However, Arneth's work opened the path for further studies along this line, and in 1914 Cooke[7] described a method for making the differential count which, though separating the neutrophiles into five distinct classes, made no further subdivisions.   He also was more specific than Arneth in his basis of classification so that the personal equation factor of error was diminished.   In using the Arneth classification, great difficulty was often encountered in deciding on the presence, and extent, of lobulation according to this author's notions; Cooke's classification led to less uncertainty by his criterion that, "if there is any band of nuclear material except a chromatin filament connecting the different parts of a nucleus, that nucleus cannot, for the purpose of the count be said to be divided."   While this method eliminated some of the objections to the Arneth count, it never came into popular use as it also was rather cumbersome, and its expression by various mathematical devices, known as weighted means, did not lend it to ready interpretation.

Schilling in 1912 translated the work of Arneth into anatomical terms and still further simplified the classification by dividing the granulocytes into the following classes:

1. Myelocytes, cells with completely round nuclei; granulocytic primary cells which are normal cells of the bone marrow and are never found in the normal blood picture.

2. Young form neutrophilic leukocytes or metamyelocytes of Pappenheim having slight nuclear indentation.

3. Band or stab forms in which no true nuclear lobulations can be distinguished but where the nucleus has a long narrow or bent form.

4. Cells with definite nuclear segmentation.

Schilling describes degenerative and regenerative shifts which he interprets as indicating the state of bone marrow function.

Let us now look into this question of the bone marrow as an organ of leukopoesis and see how an understanding of its physiology and pathology fits in with the views of Arneth, Cooke and Schilling. The polynuclear granular leukocytes under normal conditions are formed exclusively in the bone marrow. They develop by a process of maturation from the fixed reticular cells of the bone marrow and pass through the intermediate stages of myeloblast, promyelocyte, myelocyte and metamyelocyte in the bone marrow, and are delivered to the blood stream after maturation of the metamyelocytes into young leukocytes. These leukocytes become more mature in the circulating blood as shown by Ponder. The maturation here is no longer a process of cell division, but of nuclear segmentation. Finally there is a loss of motility and the cells become fragile, die and are removed from the circulation.

The entire structure of the functioning bone marrow in man is in a relatively uniform state, that is, the proportions of different levels of maturation are relatively constant.[24] The delivery of young leucocytes from the bone marrow is based on rhythmic orderly division and maturation.[25] Shaw has shown two tides in the volume of neutrophilic leukocytes delivered to the circulation, each occupying approximately twelve hours. The day tide begins in the forenoon, reaches its flood during the afternoon, and completes its ebb in the evening. The night tide starts in the evening, attains its height in the hours after midnight, and ebbs away in the early forenoon. These tides are not influenced by food, exercise or sleep. He also shows that the average daily variation in the total number of leukocytes is from 40 to 56 per cent in twenty-four hours, but that the percentage of cell types in the differential count is subject to but little variation.

Ponder aptly designates this condition of cell class stability as "the steady state" and claims that the Arneth count represents an equilibrium between cell production and cell destruction. Sabin groups leucopoetic activity into two classes, maturative and chemotactic, ordinarily one keeping pace with the other so that the proportion of young cells shows no marked fluctuation. Arneth and Schilling have shown that normally 4 to 5 per cent

of immature leukocytes are present in the blood stream and that this percentage is not influenced by the tide or so-called physiological leukocytoses.

Buchner and others endow bacterial proteins with chemotactic properties. Bacon, Noy and Eppler believe that the stimulus to increased bone marrow activity comes from the altered body protein due to hydration, and that in infection there is an increase of the substances in the body that afford the normal stimulus. Sabin sees the infecting bacteria as introducing both a chemotactic and maturative factor when a leukocytosis results, and a chemotactic factor alone when a leukopenia follows a temporary leukocytosis.

In mild infection maturation keeps pace with chemotaxis so that there is no marked increase in immature forms in the circulating blood. As the infection becomes more severe, maturation cannot keep up with the demand for leukocytes, so that an increasingly greater number of immature cells appear in the blood. Finally, in severe, overwhelming infection when all the metamyelocytes have been withdrawn from the bone marrow, the myelocytes enter the circulation and the total count drops.

With this clarified understanding of the physiology of the bone marrow and its reaction towards infection, it seems logical that a blood count which mirrors the condition in the bone marrow should interpret the severity of the infection more accurately.

In a previous paper[37] data were submitted on a series of non-infective conditions in which the total leukocyte count varied from 6000 to 15,000 and the polynuclear neutrophile count from 57 to 78 per cent, with a range of 3 to 5 per cent of immature forms. A group of secondary anemias with so-called relative leucocytosis showed from 2 to 5 per cent of immature leukocytes. A series of non-suppurative infective conditions such as acute arthritis, cervical adenitis, cholecystitis, ulcerative colitis, and so forth, gave immature counts of 7 to 12 per cent. On the other hand, in suppurative processes the immature cells amounted to more than 14 per cent. In the above study all of the members of the three immature groups of Schilling were combined under the heading of immature forms. This was done for the purpose of

simplification, both for the technician who made the count, and for the clinician who interpreted it. Although some of the sensitivity of the Schilling count is apparently lost by this procedure, we always take into consideration the degree of shifting to the left—towards the myelocytes—when interpreting the count.

A study of the value of this type of blood count in appendicitis was undertaken for two reasons; first, because in this condition the blood count is very often relied upon for aid in diagnosis, and secondly, because the count could be checked by pathologic material and its worth more readily evaluated.

TABLE 1

671 Cases of Appendicitis—Schilling Counts

HISTOLOGIC DATA	NUMBER OF CASES	TOTAL LEUKOCYTE COUNT	PERCENTAGE OF POLY-NEUTRO-PHILES	PERCENTAGE IMMATURE GRANU-LOCYTIC FORMS	AVERAGE PERCENTAGE OF IMMATURE GRANU-LOCYTIC FORMS
				per cent	
1. No evidence of active inflammation......................	277	6,400–14,600	55–83	2–8	4.3
2. Catarrhal appendicitis..........	180	8,000–18,200	67–83	7–14	10.2
3. Acute diffuse suppurative appendicitis without perforation.....	188	10,000–22,800	70–89	14–28	17.8
4. Acute diffuse suppurative appendicitis with perforation and peritonitis......................	26	11,800–30,800	69–92	32–47	39.5

DISCUSSIONS OF CASES

The material surveyed consists of 671 patients whose appendices were removed at the Newark Beth Israel Hospital from April 1, 1929 to April 1, 1930 and in whom cell counts had been done preceding the operation. These counts were reported as usual without any regard for the percentage of immature cells. All the slides used in making the counts were numbered and filed for future Schilling count. The appendices were sectioned and reported using the following classification:

1. Appendices showing no evidence of any active inflammatory process (including normal, healed, and obliterated appendices).

2. Catarrhal appendicitis, with the inflammatory process limited to the mucosa.

3. Acute diffuse suppurative appendicitis without perforation, with or without gangrene.

4. Acute diffuse suppurative appendicitis with perforation and peritonitis.

In the first group were included 277 cases (table 1). The total white count varied from 6400 to 14,600. The polyneutrophile percentage from 55 to 83. The Schilling count ranged from 2 to 8 per cent immature forms with an average of 4.3 per cent. It is interesting to note that in the cases in which the Schilling count was above 5 per cent, a temperature increase with some evidence of mild infection was clinically present, although the appendix was histologically free.

There were 180 appendices diagnosed histologically as catarrhal appendicitis studied. The total white count in this group ranged from 8000 to 18,200; polyneutrophiles 67 to 83 per cent; immature forms 7 to 14 per cent with an average of 10.2 per cent. The appendices in the patients with 14 per cent immature cell count showed beginning edema and exudation in the muscularis, but not of sufficient degree to justify a diagnosis of acute diffuse suppurative appendicitis.

One hundred eighty-eight cases of acute diffuse suppurative appendicitis without perforation and with or without gangrene showed total white cell counts ranging from 10,000 to 22,800, with polyneutrophiles from 70 to 89 per cent; immature forms 14 to 28 per cent with an average of 17.8 per cent. The immature forms seemed to follow definitely the severity of the histologic process and those cases with gangrene showed the higher counts.

Because of the fact that many of the patients with acute suppurative appendicitis with perforation and peritonitis were admitted for emergency operation, having had a blood count before entering the hospital, only twenty-six cases in this group were studied. These showed total white counts of from 11,800 to 30,800; polyneutrophiles 69 to 92 per cent; immature forms 32 to 47 per cent, with an average of 39.5 per cent.

It may not be amiss to quote here a few cases in which the blood count by the usual method was wholly misleading and in which a Schilling count would have revealed the true picture.

Case 1. A male, aged fifteen years was admitted with a history of colicky pains not associated with nausea or vomiting for an indefinite period of time. On the afternoon before admission he experienced cramp-like pains in the right lower quadrant which did not radiate and were not associated with nausea or vomiting. On admission to hospital, the temperature, pulse and respirations were normal. The abdomen was soft and flat except for resistance and tenderness in right lower quadrant. Blood count showed 10,000 white cells with 70 per cent polyneutrophiles. A clinical diagnosis of catarrhal appendicitis was made and the patient was operated upon the next morning, when an acutely inflamed appendix was removed showing acute diffuse suppuration microscopically.

This case is presented as illustrative of a group of cases of acute appendicitis in which the blood count, as done routinely, may not only be of no definite help in the diagnosis, but may even be somewhat misleading. In considering the blood count in the above case together with the almost negligible clinical data, the surgeon was warranted in waiting until the next day for routine operation. However, a Schilling count done later on the same smear showed 14 per cent immature cells among the 70 per cent of of neutrophilic granulocytes, definitely indicating a suppurative process. Had the Schilling count been taken into consideration, the patient would probably have been operated upon immediately. The histologic study in this case showed a well advanced suppuration with exudate covering the serosa. The convalescence of this patient was rather stormy.

Case 2. A male, aged 49 years, gave a history that one day before admission to the hospital he was suddenly seized with sharp pains in the right side of the abdomen after which he vomited several times. The pain persisted, keeping him awake all night. Upon admission to hospital the next morning, patient still had pain and suffered marked discomfort. Examination showed a distended abdomen with muscle spasm throughout, especially in the right lower quadrant and rebound tenderness in this region. The temperature was 100; pulse 100; and respirations 20. Blood count showed 11,800 white cells with 69 per cent polyneutrophiles. Operation was performed immediately in spite of the blood count and free pus was found in the abdomen. The appendix was markedly thickened, covered with a heavy exudate and ruptured at the midpoint. Two cigarette drains were inserted.

In this instance the blood count was entirely misleading, showing a descending line according to Gibson's and Wilson's studies, whereas a Schilling count showed 47 per cent of immature cells among the 69 per cent polyneutrophiles. Many of these immature forms were myelocytes, showing a marked shifting to the left. This should have definitely led to a diagnosis of severe infection and to the suspicion, at least, that peritonitis had already complicated the picture.

From the above study one gains some rather definite impressions as to what can be learned from this type of count. In the presence of a normal immature count, I agree with Cooke[6] that an inflammatory process in the appendix can be definitely excluded. When the immature count is below 14 per cent I feel quite certain that there will be no diffuse appendicitis and that the inflammation is limited to the mucosa and has free drainage. With immature counts of more than 14 per cent immediate operation is always recommended and as the count approaches 25 to 30 per cent, gangrene of the appendix or localized peri-appendicitis is the invariable pathologic finding. When the percentage of immature cells reaches 35 or more, the diagnosis of ruptured appendix with peritonitis is almost always correct.

Repeated counts following operation in these cases give a reliable index as to prognosis. A return towards normal in the number of immature forms indicates a subsidence of the infection. A maintained high Schilling count should be interpreted as persistence of infection, probably with a localized inflammatory process about the stump or the cecum. A rising Schilling count is of very serious prognostic import and usually indicates a peritonitis. If there is a rise in immature cells after several prior counts had shown a recession in the immature cells, it indicates some complication, and according to Reznikoff, serves to detect this complication before it is clinically apparent.

SUMMARY

The methods in vogue at the present time for interpreting the leucocyte count in the diagnosis of appendicitis are not satisfactory as evidenced by the literature. The degree of leuko-

cytosis itself is of no value due to physiological factors, such as the diurnal tide of Shaw, migration of leukocytes into the tissues, and redistribution of cells in the peripheral circulation as spoken of by Mirkin and Rachlin. The differential cell count giving the percentage of polymorphonuclear leukocytes and the relation of this percentage to the total leukocyte count, according to Gibson's standard chart and Walker's index of resistance, contributes valuable information as to the patient's resistance to infection in many instances, but cannot be interpreted into terms of pathologic lesions. The response of the body to infection through the leukocytes is a matter of bone marrow function. Sabin and others have shown that the correspondence between bone marrow and blood are relatively exact insofar as cell picture is concerned. The Schilling count is an interpretation of the blood picture in terms of bone marrow function.

CONCLUSIONS

From a study of 671 cases in which the Schilling blood picture was correlated with the histologic observations in the appendix, I conclude:

1. That the presence of a normal percentage of immature forms rules out appendicitis.

2. That an immature count of less than 14 per cent indicates a mild process, probably limited to the mucosa.

3. That an immature count of more than 14 per cent indicates a diffuse suppurative appendicitis, of increasing severity as the count approaches 30 per cent.

4. That more than 35 per cent of immature cells indicates perforation, with peritonitis.

5. That repeated counts are of definite prognostic value.

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HEMORRHAGE WITH SUDDEN DEATH IN TRACHEO-BRONCHIAL LYMPH NODE TUBERCULOSIS IN ADULTS*

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Current medical literature emphasizes the importance of tracheo-bronchial lymph node tuberculosis in adults. Many are convinced that the problem of pulmonary tuberculosis in adults arises largely from the extension of the disease to the lungs and pleurae from some focus in the nodes at the hilus. The autopsy and the roentgenogram have taught us how frequently these nodes in adults are infected with tuberculosis without the production of the disease. It is recognized that tuberculous disease in children is essentially of this type. Chandler and Preston pointed out that the great frequency of tuberculosis of the mediastinal nodes "must be accepted as an established fact, for in many series of autopsies performed on tuberculous children, the bronchial glands are affected in nearly a hundred per cent. of the cases, the tuberculous disease here being commoner than in any other part."

Tuberculosis of the tracheo-bronchial lymph nodes is more difficult of clinical demonstration in adults than in children, because of the absence of reliable physical signs. Nevertheless, Pratt and Bushnell declared that "only through the instrumentality of the lymphatic system" can there be a sufficient "collection of tubercle bacilli with their poisons" to produce tubercle formation "in the relatively immune individual." We are aware that normal lymph nodes at the hilus do not cast shadows in the roentgenogram.[3] Dense foci indicating calcium deposition are accepted universally as evidence of old tuberculous infection in

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these nodes. Opinions differ with regard to the detection in the x-ray film, of caseation in the tracheo-bronchial nodes, although evidence of caseation indicates that the tuberculous process is not arrested completely. However, I am of the opinion that dense shadows at the hilus which are irregular in contour and in the variations of density within the shadow, represent lymph nodes in which incomplete calcification is associated with caseation. These shadows are usually larger than the clear cut, sharply defined, deep density of completely calcified foci.

In children, tuberculosis of the tracheo-bronchial lymph nodes is associated frequently with rupture of these nodes into the blood vessels, pericardium, esophagus, trachea and bronchi.[4,c] Among adults, such occurrences sufficiently destructive to cause sudden death, must be rare. The literature calls attention to this possibility, but so far, reference has been found to only one other specific instance. Hartman, in 1925, reported the case of a colored woman in whom sudden death occurred by hemorrhage. The autopsy revealed the erosion of the trachea and innominate artery by unsuspected tuberculous infection of the tracheo-bronchial lymph nodes.

Three cases of sudden death in adults by hemorrhage due to the erosion of pulmonary vessels in tuberculosis of the tracheo-bronchial lymph nodes, are reported here.

Case 1. A male negro, aged thirty-three years, entered the hospital after an illness of three months, "for treatment of left spontaneous pneumothorax and pleurisy with effusion." This diagnosis had been substantiated by previous x-ray examination of the chest. No lesion in the lungs had been noted in the x-ray report, nor detected in the previous physical examination. Upon the entrance of the patient to the hospital the provisional diagnosis of pulmonary tuberculosis was made. The temperature was 98.6°F., the pulse 84 and the respiration 20. There had been a loss of weight from 175 pounds to 159 pounds within a year. The chief complaints were cough with expectoration, hoarseness, headache, pain in the left chest, weakness and easy fatigability. The admission date was August 15, 1927, 2:10 p.m. The patient was given the routine care for new patients and his condition appeared "fairly good." Hemorrhage from the nose and mouth began at 2:30 a.m., August 16, twelve hours after admission. The patient had arisen and gone to the lavatory. Palliative treatment failed to check the flow of blood and in twenty minutes he was dead.

At the autopsy the organs were found bloodless. Easily torn fibrous adhesions were present between the visceral, parietal and interlobar pleura on the right. Gross examination of the right lung revealed no pathological lesion. The left lung was described as follows: The left lung fills the left half of the chest completely, is almost black in color, friable, and has the appearance, weight and consistency of liver. Only a very few fibrinous, easily torn, pleural adhesions are present. The lung is several times heavier than the right and pieces of it sink in water. The organ cuts like liver. The cut section possesses a porous rubber sponge-like appearance. The upper lobe and the upper portion of the lower lobe are dark scarlet. The remainder of the lower lobe is black and densely solid without porosity. The blood vessels are engorged. Their walls are bright red. Repeated section and dissection fail to reveal any primary site of pathology. An incision through the hilum discloses an encapsulated, confluent mass of caseous and calcareous lymph nodes. This focus occupies the angle between the trachea and the left bronchus and has a diameter of 3 cm. The nodes included within the thick, fibrous capsule have lost their identity and form a single degenerated, cheesy, calcareous mass which is easily shelled out. The lower border of the capsule is in juxtaposition to the left descending bronchus and branch of the pulmonary artery. An opening large enough to admit the tip of the index finger, connects the encapsulated mass with the lumens of artery and bronchus. No gross pathological lesions were found elsewhere. Microscopical examination of the apex of the right lung revealed miliary tubercles. These were not numerous and consisted largely of lymphoid cells interspersed with fibrous stroma and a few polyhedral cells. Only an occasional center appeared necrotic.

Case 2. A male negro, aged thirty-five years, entered the hospital on May 24, 1928, complaining of cough, spitting of blood, night sweats, and loss of weight. Temperature ranged from 97°F. to 103°F., pulse 80 to 120, respiration 20 to 35. Physical examination elicited increased fremitus, dullness, whispered pectoriloquy and whispered voice. Breath sounds were broncho-vesicular. Râles were present. The x-ray showed infiltrations and consolidations with a large cavity in the left apex. Four out of eight sputa were positive. The blood Wassermann was negative. The course of the disease was associated with repeated hemoptysis. Death followed a hemorrhage on June 11, 1928.

At autopsy the lungs were described as follows: The hilus lymph nodes are greatly increased in size, are soft, black and caseous. The largest is 4 cm. long. The left lung is adherent at the apex and base. The lung is soft, crepitates and floats. On cut section, fibrous scars appear in the apex. Small, discrete, caseous areas are scattered throughout all lobes. Numerous, large, caseous lymph nodes appear along the course of the bronchi and blood vessels. The right lung is free, pink, soft and crepitates. On cut section there is emphysema in the upper lobe. The middle and lower lobes are red and moist, with small, raised, irregular, scarlet areas about the bronchi. The nodes along the bronchi and blood vessels are likewise large and caseous. Along the course of the bronchus and pulmonary artery of the right middle lobe, several of these caseous nodes appear. One has eroded through the walls of both bronchus and artery.

Case 3. A male negro, aged thirty-seven years, was admitted to the Hospital January 14, 1928. He had "caught cold" in January, 1927. Since then he had complained of cough, shortness of breath and "spitting of blood many times." *Mycobacterium tuberculosis* were found in the sputum; the blood Wassermann showed a two plus reaction. The x-ray disclosed numerous infiltrations and irregular areas of consolidation throughout the upper portions of both lung fields. On November 20, 1928, he left his ward without permission, jumped a fence, and died in ten minutes from pulmonary hemorrhage.

The autopsy revealed a moderately advanced pulmonary tuberculosis, bilateral tuberculosis of the mediastinal lymph nodes and erosion of a branch of the pulmonary artery in the right middle lobe, by a caseous lymph node.

Three cases of sudden death in adults by hemorrhage due to the erosion of pulmonary vessels by tuberculosis of the tracheobronchial lymph nodes, are sufficient to call attention to the possibility of such an outcome. The deaths reported occurred within fifteen months. In the first patient no definite, clinical evidence of pulmonary tuberculosis had been observed. There had been no previous spitting of blood. In the second and third patients, pulmonary tuberculosis had been demonstrable and was associated with blood in the sputum. Neither had been seriously ill, however, and it is evident that for them, the infection in the lymph nodes was more serious than the pulmonary invasion.

It is striking that all four cases have occurred in negroes. This raises again the question of the character of the tuberculous infection in negro adults. Does tuberculosis in negroes tend to conform to the juvenile type, rather than the adult type usually observed in whites? This much may be stated, that in a series of over 200 autopsies upon adult male negroes dying of tuberculosis, gross evidence of massive infection in the tracheo-bronchial lymph nodes, has been observed in every case.

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THE INFLAMMATORY NATURE OF NODULAR GOITRE AS A CHRONIC THYROIDITIS*

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The purpose of this paper is to present the conception that many of the nodular thyroid glands should be considered as being inflammatory rather than as tumors. This is a part of a critical study of 1000 thyroid glands over a period of eight years. These cases represent the routine hospital admissions for thyroid diseases from the fringe of the goitrous region in central Pennsylvania. The cases include all forms of thyroid disease with the nodular types making up more than one-fourth of the group.

There is no doubt that the pathology of the thyroid gland, while it follows the laws of reactions elsewhere in the body, has its own individualities. Applying a general knowledge of histological pathology to the first hundred cases one studies and following the general outline of textbooks, one considers at first many cases of tumor and too large a number, possibly malignant. Then, as more and more sections taken from different parts of the same gland are studied, the picture with its wide variations becomes more confusing. It gradually dawns on one that these reactions are not all hyperplasias but something else. What this something else may be becomes quite clear when all structures in the glands are taken into consideration. From this standpoint the pictures are those of inflammation.

It is necessary to define my conception of hypertrophy, hyperplasia and inflammation. An hypertrophy is an increase in numbers of cells and size of a part associated with an increase in function. An hyperplasia is an increase in numbers of cells and size

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of a part without necessarily increase in function. Indeed, there is often the reverse in hyperplasia—a decrease in function. An inflammation is a reaction on the part of the tissue to a stimulus. The stimulus may be anything but must be present. The reactions follow definite laws of transudation, exudation, degeneration, regeneration and hyperplasia.

I realize that large numbers of cases give a valuable experience but Osler once said ten beds well studied are worth more than the prefunctory rounds of a hundred. This study is based on only one thousand cases but it represents the milling over of available data over a period of ten years. A complete redigest of the histories, gross specimens and microscopic slides was made after the conceptions as set forth in the paper were reached. This has afforded a uniformity of conclusion. Every gross specimen which had been labelled and stored was reviewed and again briefly described to be compared with the full report. All available microscopic slides were restudied. These briefs were then put together and the tabulations made at one time. The conceptions, of course, were the result of gradual rearrangement of ideas as the years went by.

In reviewing textbooks and the literature a great deal of confusion exists as to just what is meant by the types enumerated and each man who has studied the question has devised a nomenclature to meet his needs. Others have combined classifications and as a result hardly two authors agree as to either clinical or pathological classifications. In addition, errors creep in and are perpetuated. For example, Riedel described his firm type of thyroid as "eisenharte" (iron hard). This is variously described as Riedel's thyroiditis, Riedel's thyroid tumor and worst of all, as Eisenhart's strumitis.

The pathology of the thyroid gland as seen in operative specimens, has been greatly altered by the widespread use of iodine. This factor must be considered from now on in any study of the relationship of the actual pathology and the disease entity as compared with the years before iodine therapy. Fortunately, most of my own work was carried on before the use of iodine, and represents, therefore, the actual and not the altered pathology of the gland.

TABLE 1
PATHOLOGICAL CLASSIFICATION OF THYROID DISEASES

I. Normal thyroid.   No pathological changes.
II. Anomalies.
    1. Absent.
    2. Accessory.
III. Atrophy.
    1. Congenital deficiency (Type: cretinism and myxedema).
    2. Senile.
IV. Metamorphoses.   Alone or part of complex reactions.
    1. Congestion.
    2. Hemorrhage.
    3. Colloid.
    4. Hyalin.
    5. Amyloid.
    6. Necroses.
    7. Cloudy swelling.
    8. Calcification.
    9. Ossification.
V. Hypertrophy (colloid goitre).   Increase in size and function.
    A. Primary hypertrophy (in response to function).
        1. Adolescent.
        2. Pregnancy.
        3. Trophic.
        4. Congenital.
    B. Primary hypertrophy with secondary complex reaction.
        1. Hypertrophy with inflammation.
        2. Hypertrophy with tumor formation.
VI. Inflammations (includes nodular thyroids, many so-called toxic adenomas).
    All inflammatory reactions.
    A. Acute.
        1. Parenchymatous (exophthalmic goitre).
        2. Degenerative (infectious entities).
        3. Exudative (infectious origin).
        4. Suppurative (abscess and pyemia).
        5. Gangrenous (post-traumatic).
    B. Chronic (nodular).
        1. Productive (enlarged glands, acinal and trabecular increases).
            a. Cellular.
            b. Fibrous.
            c. Colloid.
            d. Cystic.
            e. Calcareous.
            f. Osseous.

TABLE 1—*Concluded*

VI. Inflammations—*Concluded*
    2. Contractive (small glands, atrophy, fibrous contraction, calcium and bone).
        a. Fibrous.
        b. Cystic
        c. Calcareous.
        d. Osseous.
   C. Specific.
    1. Tuberculous.
    2. Syphilitic.
VII. Neoplasm.
   A. Benign.
    1. Adenoma (acinal, interacinal (Wolfler) epithelial origin. Pure tumors.)
        a. Single.
        b. Multiple.
        c. Cystic.
        d. Colloid.
        e. Degenerative.
        f. Foetal.
    2. Adenoma with thyroiditis.
    3. Fibroma (trabecular connective tissue origin).
    4. Teratoma and dermoid cysts.
   B. Malignant.
    1. Carcinoma.
        a. Simplex.
        b. Adeno.
        c. Papillary.
    2. Sarcoma.
        a. Round and spindle cells.
        b. Endothelioma.
        c. Perithelioma.
        d. Myxosarcoma.
VIII. Parasitic cysts (Echinococcus).

With this explanation and in order to fix definitely the type of thyroid with which this paper is concerned, it is necessary to present a pathological classification (table 1). I do not wish further to cloud the situation, of which I have just complained, but desire to present the beginning of what might be taken for the structure of a classification. Any complete pathological study must be detailed because of the different types of tissue concerned. This

does not preclude a more simplified form for clinical usage which I have also included as a guide. I know that both these lists can be improved and I have changed them many times. They offer, however, a very satisfactory means of definitely cataloging the same general types of cases. A more detailed report on this classification will appear later.

The present paper is concerned with classification of chronic thyroiditis either productive or contractive. Many of the nodular types of goitre, about half of which are toxic, are not true tumors but are phases of an inflammatory reaction. It is true that areas of epithelial hyperplasia are to be found in certain parts of the gland but the predominating changes in the supporting structure are inflammatory with epithelial regeneration and degeneration and associated accumulations of colloid. To my mind the accumulated colloid is an innocuous vehicle for the storage and dilution of the thyroid active principles just as mucus is an innocuous lubricant and vehicle. While the thyroid gland is a so-called ductless gland, in reality the relationship of the acinal epithelium histologically with the surrounding capillaries makes it almost a sponge squeezing its product into the blood stream. It must have something to withhold its activity during periods of overproduction as in the case of the gall bladder for the liver.

The colloid acts as the material of storage. When this delicate mechanism is interfered with either by demands of function or by an outside stimulus changes occur in the stroma as a result of the stimulus and in the epithelium as an activity or degeneration and clinical symptoms result. This mechanism originated within or without the thyroid gland, fits in perfectly with that of other so-called ductless glands such as the adrenal.

In this series the different types of glands were divided according to the chart. Twenty-nine (3.3 per cent) glands were considered pathological normals, one hundred twenty-two (14.2 per cent) were pure primary hypertrophies, (Marine's type of colloid goitre), one hundred fifty-one (17.6 per cent) were hypertrophy with secondary thyroiditis. The pathological changes began as a pure hypertrophy and progressed through stages of inflammatory reaction. These are the nodular types of chronic goitres of long

slightly toxic condition, the toxic types diagnosed clinically as toxic adenoma, falling into the inflammatory thyroiditis group. I would, therefore, like to suggest that the clinical term adenoma is a misnomer, is confusing and should be dropped from consideration as rapidly as possible.

As has been true in the history of nephritis, so with goitre the surgeon and internist cannot be expected to use clinically a complicated pathological classification. I, therefore, propose, subject to future modification, a short clinical classification based on the pathological conceptions outlined in this paper (table 3).

TABLE 3

CLINICAL CLASSIFICATION OF THYROID DISEASES

(Simplified from pathological classification)

The pathological state to be applied to clinical usage only where it is definite and elucidative.

All clinical cases to be divided into:
    Group I: Toxic goitre.
    Group II: Non-toxic goitre.
and subdivided into subgroups under either group:
    A. Thyroid atrophy.
    B. Thyroid hypertrophy.
    C. Thyroiditis, acute, chronic or specific.
    D. Neoplasms benign or malignant.

This places all cases into one of two main groups—toxic or non-toxic goitre. It further distributes them under four main types: (A) Thyroid atrophy, the cretin and hypothyroid group; (B) Thryoid hypertrophy, the single colloid goitres, Marine's type which are certainly not surgical but medical; (C) Thyroiditis, acute or chronic. The true exophthalmic goitres are included under acute throiditis. In this group are included a large number of nodular thyroids uniformly involving both lobes, occurring most often in females, most frequently between thirty and forty, about equally divided between toxic and non-toxic but giving other thyroid symptoms. (D) The true tumors, benign, as adenoma, and malignant, as carcinoma.

The other rare forms of thyroid disease such as tuberculosis,

true syphilis, echinococcus cyst, Chagas parasites, Riedel's thyroid, acute gangrenous thyroiditis are special entities and as such will probably not be accurately diagnosed clinically or pathologically until completely studied.

This shorter clinical grouping offers a definite place for each pathological type of thyroid and is more accurate than the present method of placing the majority in a group most favored by the particular clinician.   In this series in the thyroiditis group alone, twenty-six different clinical diagnoses were made by the surgical staff with the vastly predominating group falling under multiple adenoma and such terms as substernal goitre, multiple colloid goitre, colloid adenoma, adenomatous goitre, and so forth, were made.   With this multiplicity of terms it is obviously impossible to arrive at any accurate conclusion on the group.   On the other hand, if one clinician in speaking of toxic adenoma and another of exophthalmic goitre mean the same type, the confusion is just as bad.

The clinicial picture of chronic thyroiditis varies.   The gland is always enlarged above the normal, usually uniformly.   Some parts are frequently more involved than others and in the substernal type most of the enlargement may be below the sternum. The gland is predominantly nodular, the resistance is increased over that of simple hypertrophy.   In the cystic types fluctuation may be apparent and in the smaller contractive forms the denseness of the calcareous and bony changes may increase the resistance.

The gland in the gross is increased in size, each lobe weighing from 45 to 125 grams.   The normal thyroid contour is preserved but the surface is almost always nodular.   The capsule is usually thin and smooth but it may be very thick with torn tags as a result of removal.   The blood vessels are collapsed but in some cases are very thick and stand out prominently on the surface. This is associated with heavy bands of connective tissue penetrating into the gland along the trabecular lines.   The consistency of the gland varies from soft to quite firm, dependent upon the amount of fibrosis present.

The cut surface is always lobulated as a result of fibrous tra-

beculations. The even colloid mosaic of the normal and hypertrophied gland is upset by the irregular fibrosis. The colloid will bulge on the surface in a pebbly way or larger areas may be cystic. Large circumscribed areas of clear or blood tinged colloid may be present. This may be quite fluid and appear as a yellowish-white material. Where areas of acinal hyperplasia are of any size the surface will show spots of fleshy consistence. The calcareous and osserous areas will of course be apparent. In the small contractive types the surface may be very firm and almost beefy. These are to be distinguished from the uniform tumor cases. The characteristic surface shows lobulations of fibrous traceculae sharply defining areas of accumulated colloid.

The microscopic picture will vary with each gland dependent upon the amount and the duration of reaction. The trabeculations will show all types of connective tissue activity from round cells, plasma cells, fibroblasts to heavy hyalinized strands. The latter may be infiltrated with calcium. The bony changes never show any lamella but when decalcified are homogenous masses of structureless material.

The glands show a great variety of change, differences in size, some widely distended with colloid, others contracted. Areas of acinal hyperplasia are seen in the reduplication of lining cells. These are often masses of cells and some areas of new acinal formation but not the uniform hyperplasia of the definite tumors. There is often variation in the picture of different parts of the same gland. Mitotic figures are not the rule in the acinal cells but they may be present. The acini may be very compact and appear as syncytial masses or giant cells. These are not tuberculous but have malignant potentials.

Accumulations of round cells as masses or as a general increase are common. In the very heavy proliferation of these the similarity to a leukemic change is striking. It has always seemed, however, like a specific bacterial reaction. The perivascular spaces at times are filled with round cells.

The acini are often separated into groups, widely distended with colloid. These are walled off by heavy trabeculae of fibrous tissue but without any fibrosis whatsoever between the affected acini.

The staining reactions of the colloid varies within wide limits. This has all been carefully described.

Congestion of blood vessels is not the rule but in some cases this is marked with areas of hemorrhage. I usually feel that most of this is traumatic.

In the dense scar area there are spaces which have contained fatty acid crystals.

SUMMARY

Following the combined study of histories, gross specimens and microscopic slides over a period of ten years, the opinion is expressed that a large proportion of the nodular thyroid glands, toxic and non-toxic, show the pathological evidences of chronic productive and contractive thyroiditis rather than of adenoma.

The true adenomata in the group follow the pathological conceptions of tumor elsewhere in the body.

A pathological classification is proposed based upon the conception that many of the nodular types of glands are inflammatory and not tumorous.

The incidence of these types under these conditions was studied and tabulated in 1000 cases.

A short clinical classification is offered.

THE KLINE PRECIPITATION REACTION AS AN ADJUNCT TO THE COMPLEMENT-FIXATION TEST IN THE SEROLOGICAL STUDY OF SYPHILIS*

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The status of the tests for the precipitation reaction in the serological study of syphilis has been the subject of extensive and even vehement discussion in the literature of recent years. Originally they were advocated as a substitute for the more laborious and time-consumping complement-fixation test, which the proponents of precipitation tests demanded should be discarded as obsolete. From the ensuing extended investigations have emerged certain rather clear-cut conceptions concerning the status of the precipitation reaction in syphilis.

Briefly recapitulated, these are as follows:

1. The precipitation reaction is completed much more rapidly than the complement-fixation reaction.

2. While technically more simple with regard to the reagents and apparatus required, these tests are not proportionately more simple as far as the mechanism and phenomena involved and, therefore, are equally open to errors of technical origin.

3. Because of the above fact, performance of the Kahn test, as a prototype of precipitation reactions in syphilis, as has been emphasized by many and as later admitted by Kahn, should be left to those properly trained in clinical laboratory methods.

4. The proper reading and interpretation of "border-line" precipitation reactions requires a marked degree of serological training and experience. These tests, therefore, are not suited for infrequent use as office procedures by those whose serological

training has been superficial or inadequate and whose experience in such matters has been limited or sporadic.

In this connection the report of the League of Nations Conference may be cited, in which it is stated that the conference

"desires to emphasize the fact that, no less than the complement-fixation tests, these flocculation methods are, despite their apparent simplicity, extremely sensitive to the slightest difference in experimental conditions and subject to as many sources of error, in connection both with the execution of the test and the reading and interpretation of the results, so that they must be placed only in the hands of specially trained serologists."

5. The results of numerous and extended investigations have shown that, as could be expected from the inherent principles underlying the production of serological phenomena in syphilis, some sera will react at one time to the precipitation and not to the complement fixation test and vice versa. From this it follows that: (a) the two tests supplement one another; (b) confirmation of a weak (plus-minus) precipitation reaction is afforded by a coincident positive complement-fixation reaction and vice versa; and, therefore both tests should be used in the serological study of syphilis, one complementing the other and so increasing their joint significance and reliability.

There can be little question that the work of Kahn and his associates has added much to the technical development of the precipitation reaction in syphilis and has stimulated the development of a number of such methods varying in detail. The determination of their suitability as adjuncts to the complement fixation test is of interest and importance.

The purpose of this communication is to present the results of a study of the test proposed by Kline and Young which is not only as rapidly performed as the Kahn test but has the advantage of being technically even more simple.

The present communication supplements and corroborates the conclusions advanced in a previous report upon the same subject and embodies a comparative analysis of 2623 tests, all being routine examinations during 1929 of unselected sera.

The sera were from three sources: (1) Patients from all services in the hospital submitted for routine examination; (2) specimens

from private patients not in the hospital; (3) specimens from the Veneral Disease Clinic of the Municipal Hospital.

Unless the exact nature as well as the reliability of each is known, comparison or analysis of the results of serological procedures is not only unprofitable but may be misleading.

The routine complement-fixation test used in these laboratories is the six-tube quantitative test described by Kolmer the delicacy, reliability, and relative specificity of which in competent hands is no longer a matter of debate.

The Kline test was performed as directed by Dr. Kline after he had very kindly demonstrated his method. The necessary reagents for both tests were made in these laboratories.

Since the original publication Kline has proposed a new antigen as well as "very delicate" and "presumptive" procedures

While it is true that, at times, the clinical diagnosis of syphilis is impossible because of the absence or paucity of clear-cut clinical data and in such instances must rest upon the results of dependable serologic procedures performed by competent workers, it is also true that, whenever possible, serodiagnosis should be confirmed by clinical and therapeutic evidence.

The introduction of so-called "very delicate" and "presumptive" procedures, conveying as it may to the clinician at large a suggestion of absolute specificity incompatible with the mechanism of both complement-fixation and precipitation reactions as these are at present understood, deserves careful consideration. There is a limit to which the delicacy of serological procedures may be advanced without introducing the possibility of false positive reactions the dangerous potentialities of which as regards the patient require no comment.

Moreover, as the purpose of the study was to discuss the suitability of the Kline test under general conditions, the original technic was adhered to and innovations not introduced.

As stated 2623 sera were tested with the following results:

Negative to both tests............................ 2115 or 80 per cent
Negative to Kolmer test........................... 2192 or 83 per cent
Negative to Kline test............................ 2212 or 84 per cent
Positive to Kolmer test........................... 383 or 14 per cent
Positive to Kline test............................ 462 or 16 per cent

While there was thus a very close agreement of both procedures with regard to the negative sera, the discrepancy in both negative and positive reactions, if hastily considered alone, could be accepted as an indication of increased sensitivity on the part of the Kline test. Considered in the light of the extended investigations on precipitation reactions in general, it simply exemplifies what is now widely recognized: that a serum on a single test may be positive to one and negative to the other procedure or vice versa. It thus emphasizes the value of their coincident use.

Moreover, while the Kline test gave seventy-nine more positive reactions than the Kolmer test, forty-five or 57 per cent, of these were plus-minus reactions which, in the absence of definite knowledge of syphilis in the patient in question, must be regarded as without diagnostic significance and as suggesting only the advisability of further study.

The remaining thirty-four positive reactions were clear-cut and indisputable (two plus or more).

In 252 of the positive reactions both tests agreed. While this agreement was absolute with regard to positivity, it was not absolute with regard to the *degree* of positivity, there being numerous Kolmer reactions of definite strength (44440; 44300; 44000, and so forth) coupled with Kline reactions of plus one, plus two, and plus-minus, as well as a number of plus two to plus four Kline reactions coupled with weakly or moderately positive Kolmer reactions (fixation only in the first or second tube).

While it is impossible to evaluate acurately this phase of the study in the absence of equally complete clinical data, the impression gained was that the Kolmer test, if positive, was definitely so, the reaction possessing a definite significance, whereas the Kline test, when weakly positive would have been difficult of clinical interpretation in the unknown case.

It was not uncommon, on the other hand, in the presence of syphilis under treatment, to find the Kline test remaining positive in some degree longer than the complement-fixation test, a phenomenon generally recognized in connection with acceptable precipitation procedures.

The absolute disagreements occurring in 250 sera were as indicated in table 1 which, for brevity's sake, shows only the first fifty sera, anticomplementary sera being omitted.

There were thirty-six, or 1 per cent, anticomplementary reac-

TABLE 1

Illustrating Nature of Opposing Reactions

KOLMER TEST	KLINE TEST	REMARKS	KOLMER TEST	KLINE TEST	REMARKS
00000	plus 1	Treated	30000	0	Unknown
00000	plus 1	Treated	00000	plus-minus	Unknown
44100	0	Lues	44400	0	Unknown
43000	0	Lues	40000	0	Unknown
44400	0	Unknown	00000	plus-minus	Unknown
42000	0	Unknown	44441	0	Lues
40000	0	Early lesion	40000	0	Lues
0	plus 1	Treated	00000	plus-minus	Treated
0	plus 3	Unknown	00000	plus 2	Treated
40000	0	Donor	00000	plus 3	Unknown
00000	plus 1	Treated	44400	0	Unknown
00000	plus 2	Treated	44400	0	Unknown
44400	0	Spinal lues	01400	0	Primary lesion
44000	0	Unknown	21000	0	Unknown
00000	plus-minus	Unknown	00000	plus 1	Unknown
00000	plus 1	Unknown	43200	0	Unknown
00000	plus 2	Unknown	00000	plus 2	Unknown
44400	0	Unknown	00000	plus 2	Unknown
44440	0	Unknown	44400	0	Lues
04400	0	Unknown	00000	plus 2	Lues
04443	0	Treated	00000	plus 2	Lues
21000	0	Treated	00000	plus-minus	Treated
00000	plus-minus	Treated	00000	plus 1	Treated
40000	0	Unknown	30000	0	Unknown
44400	0	Unknown	44400	0	Lues

tions in all of which Kline test readings were possible, the positive readings in twenty-three sera being shown in table 2.

It may be remarked here that a small number of sera were encountered in which the Kline test could not be read but these were not included in the series.

The data thus summarized suggest that the Kline precipitation

test parallels quite closely the results secured with the Kolmer test and that it is quite suitable as an adjunct to the complement-fixation test in the serological study of syphilis.

The experience accruing from an ever-widening use of the Kolmer test during the past eight years, is quite conclusive that when the Kolmer test is done in strict compliance with Kolmer's directions a definitely positive reaction does not occur in the absence of syphilis or yaws.

The close agreement of the Kline test with the Kolmer test suggests that it also has a high degree of relative specificity. Proof, of this, however, must come largely from clinical evidence and to this end the sera from the Venereal Disease Clinic, for which clinical data is available, are separately considered.

TABLE 2

POSITIVE KLINE READINGS WITH TWENTY-THREE ANTICOMPLEMENTARY SERA

NUMBER OF SERA	KLINE READINGS
2	plus-minus
1	plus 1
4	plus 2
1	plus 3
15	plus 4

Of 514 such sera, 147 were Kolmer positive and 148 Kline positive, an incidence of 28 per cent for each test.

Of the 148 Kline positive sixteen, or 11 per cent, were plus-minus reactions which, in the case of known syphilitic sera, were of definite significance. The majority of the Kolmer reactions in the same sera gave fixation in the first two tubes or, if in one tube only, a reaction seldom below plus three.

Three per cent, or 19 sera, were anticomplementary in all of which Kline readings were definite and readable.

The Kolmer test was negative in 375 sera, or 72 per cent, while 392, or 76 per cent, were negative to the Kline test. These figures do not imply a corresponding lack of delicacy in the Kline test but again illustrate the fact that a serum may be negative to one procedure and positive to another at a particular time.

However, the actual disagreements, which are shown in table 3 are in favor of the delicacy of the Kolmer test.

TABLE 3

Disagreements in Known Syphilitic Sera

(Antieomplementary sera omitted)

KOLMER TEST	KLINE TEST	REMARKS	KOLMER TEST	KLINE TEST	REMARKS
00000	plus 1	Treated	44410	0	Treated
00000	plus 1	Treated	00000	plus 2	Treated
00000	plus 1	Treated	00000	plus-minus	Treated
40000	0	Treated	00000	plus 3	Treated
00000	plus 2	Treated	00000	plus-minus	Treated
00000	plus 1	Treated	44400	0	Treated
44400	0	Spinal lues	44443	0	Treated
44000	0	Treated	44400	0	Treated
04400	0	Secondaries	00000	plus-minus	Treated
44444	0	Lues	44400	0	Treated
00000	plus-minus	Treated	44400	0	Treated
00000	plus-minus	Treated	00000	plus 2	Treated
00000	plus 2	Treated	00000	plus-minus	Treated
44400	0	Treated	44300	0	Treated
44400	0	Lesion	44410	0	Treated
00000	plus-minus	Treated	40000	0	Treated
00000	plus 1	Treated	00000	plus 2	Treated
01400	0	Treated	00000	plus 1	Treated
44400	0	Treated	44400	0	Treated
44000	0	Treated	00000	plus-minus	Treated
43200	0	Treated	00000	plus 4	Treated
00000	plus 2	Treated	00000	plus-minus	Treated
00000	plus 2	Treated	44400	0	Treated
00000	plus-minus	Treated	40000	0	Treated
44400	plus-minus	Treated	10000	0	Treated
44000	0	Treated	44400	0	Treated
44100	0	Treated	00000	plus-minus	Treated
00000	plus-minus	Treated	04100	0	Treated
20000	0	Treated	04400	0	Treated
42000	0	Treated	44000	0	Treated
44400	0	Treated	44000	0	Treated
44000	0	Treated	44400	0	Treated
00000	plus 2	Treated	44000	0	Treated
00000	plus-minus	Treated			

SUMMARY

The analysis of a comparative series of 2623 parallel Kolmer complement-fixation and Kline precipitation tests indicates that the Kline technic is a suitable method for the performance of the precipitation test in conjunction with the complement-fixation test in the serological study of syphilis.

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THE REACTION OF THE MENINGES TO THERAPEUTIC SERUM*

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INTRODUCTION

Serum sickness, in its various manifestations, has become a very familiar entity during the past twenty-five years. The reaction of the skin, mucous membranes, joints and lymphatics, following the injection of prophylactic and therapeutic sera has been dealt with at great length in the literature. Kennedy stated that in American literature no mention had ever been made of the effect of injected sera on the structures of the nervous system. In this paper I report certain observations relating to the effect of anti-meningococcal serum on the meninges, briefly review the foreign literature on the subject, and attempt to point out the practical application of the observed facts.

CASE REPORT

Miss M. C., a laboratory technician, was engaged in the routine study of spinal fluid from a patient suffering with meningococcal meningitis. The fluid unexpectedly spurted from a syringe, striking the technician in the face and spattering in the eyes and nose. The danger of the situation was realized and steps taken to prevent the development of infection. Three days later, vague joint pains were complained of and six days after exposure, headache and general malaise were reported. During this day, the temperature rose from 98.6° to 104° and the patient was admitted to the hospital. Headache, projectile vomiting, stiffness of the neck and a positive Kernig sign soon developed. Lumbar puncture revealed a turbid fluid under pressure. It was found to contain 3430 cells and a Gram-negative diplococcus which was later proved by cultural studies to be a meningococcus. The same organism was recovered from

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the blood stream. Antimeningococcal serum was administered by vein and intraspinally. A total of 45 cc. of serum was given intravenously and a total of 230 cc. intraspinally. The product of a well known manufacturer was used in conjunction with serum from the New York City Department of Health, secured through the courtesy of Dr. Josephine Neal. The clinical course of the disease was favorable. The cell count fell to 91 and organisms disappeared from the fluid. The last of twelve daily injections of serum was given on March fourteenth.

Six days later, March 20, there was a recurrence of headache and general malaise. The spinal fluid cell count rose to 391, and meningococci were demonstrated in the fluid. Fifteen cubic centimeters of sera were given intraspinally following the withdrawal of 25 cc. of fluid. Within one hour after the injection, the patient began to be extremely restless and quickly lapsed into unconsciousness. The back gradually became stiff and opisthotonos was very marked. The pupils were irregular and pupillary reactions to light were very sluggish. The preexisting papilledema did not increase. Lateral nystagmus was constantly present and the power of ocular convergence was lost. A high pitched nasal cry was emitted at frequent intervals. There was no cyanosis and no marked variation in pulse, temperature or respiration. The extremities jerked about in continuous, convulsive movements. In semi-rational moments the patient complained bitterly of lancinating headache, pains in the back and especially in the lower extremities. There was complete loss of voluntary sphincter control. Careful examination failed to reveal any involvement of joints or lymph glands. Lumbar puncture was performed. The pressure was approximately the same as that of the preceding punctures (325 mm. of water). The fluid contained 4536 cells but no organisms. No serum was administered. There was no improvement following withdrawal of fluid. The acute symptoms persisted about sixty hours. Four days after the onset of the paroxysm, the cell count had fallen to 291 and the patient was conscious. It was noted at this point that the spinal fluid containing 291 cells was just as turbid as that which had contained 3000 cells. This turbidity was observed for several days and finally disappeared; that is, the degree of turbidity of the fluid and the cell count seemed to be once more in proportion. In an attempt at desensitization, 0.1 cc. of serum was given intravenously. Within an hour the previously described symptoms of coma, convulsions and opisthotonos occurred but in less severe form. Three hours later, these severe symptoms had subsided. There was no further administration of serum by any route. After a prolonged convalescence, the patient recovered entirely and returned to duty.

REVIEW OF LITERATURE

Kennedy reported one case with fulminating cerebral symptoms and five cases of peripheral nerve involvement, following the

administration of serum. The description of his first case coincides closely with my observations. He concluded that the symptoms might have been due either to the toxicity of the serum or to an urticarial edema of the neural tissue. He felt that the latter view was the most tenable.

Goldman recently established the entity of serum meningitis on a firm experimental basis. He referred to another type of meningeal reaction which might be anaphylatic but did not describe the reaction in detail.

Hutinel reported three cases of a severe meningeal type of reaction after intrathecal injection of serum. The number of injections varied from three to twelve and the time between the injections from three days to six weeks. Coma and opisthotonos were pronounced and in all three instances death ensued. Two cases were complicated by tuberculous meningitis. The author felt that the reactions were due to the direct action of the serum on the meninges with the production of urticaria and edema of the meninges.

Delahet reported one case and mentioned five others which exhibited convulsions and coma, the symptoms of which he grouped under the term *choc bulbaire*. In his personal case the reaction followed the intravenous injection of a small amount of serum. The meningeal symptoms recurred at intervals even when no serum was given. The reducing substance of the fluid was much increased. The author regarded the reactions as due to the irritation of the meninges from the formation of precipitins.

Ker described the increased glucose content of the fluid as a diagnostic factor in aseptic meningitis. He did not mention the type of reaction herewith reported.

Longcope referred to cases reported by Grysez and Dupuich and Flandin. He felt that some type of immediate reaction might occur on the surface of the meninges which would give rise to the described symptoms.

Rolleston mentioned emphatically the danger of severe reaction following intrathecal injection. He referred to the work of Netter and Debre, and thought the condition was the same as that described by Dopter under the term, "Seric Meningitis."

Auer reviewed the subject of fatal reactions from intrathecal injections. He felt that the speed of the reported reactions as well as the clinical symptoms indicated increased pressure as the *modus operandi*.

Mackenzie discussed the repeated demonstration of specific precipitins for horse serum in injected individuals. He stated that in severe serum disease, the titer of circulating precipitin was high and those individuals who were insusceptible to serum disease were poor precipitin formers and suggested that the union of antigen and antibody might play a large part in serum reactions.

DISCUSSION

It is evident that the intrathecal injection of foreign serums may result in two different types of reaction. The first is the ordinary response of the meninges to the injection of any foreign substance. It is marked by an increase of total protein and of cell count. The clinical symptoms are not severe and subside quickly. It is apparent that this serum or aseptic meningitis must occur quite regularly in the treatment of tetanus, neurosyphilis and meningitis. Goldman's summary of this subject is excellent.

The type of reaction exemplified by the case herewith reported presents an entirely different and highly individual picture. It was explosive in character and was marked by severe and terrifying symptoms. It was extremely persistent and might even have lead to death. The reaction described does not show any of the usual characteristics of immediate generalized serum reactions in man. There was no evidence of bronchospasm with resulting cyanosis and dyspnoea, nor were there any evidences of splanchnic dilatation and fall of blood pressure. All of the observed phenomena pointed to meningeal irritation of a severe nature as the underlying factor.

One is forced to conclude in a general way that during the process of treatment, a localized allergy involving the tissues of the nervous system alone developed. Further evidence of this fact is supplied by the repetition of the reaction following the injection of a very small quantity of serum (0.1 cc.) intravenously. The amount of serum reaching the nervous system from this

injection must have been very minute, yet it was sufficient to excite the same reaction as produced by intrathecal administration. This interesting phenomenon has been previously observed and described by Chiray as the "reflex sign of pyocephaly."

Rolleston recognized the dangers of intravenous injection following intraspinal medication and stated that even an attempt at desensitization might be hazardous as indeed it proved to be in my case.

The rather peculiar turbidity of the fluid I observed offers field for speculation. The appearance varied somewhat from the yellowish gray spinal fluid which is turbid as the result of the presence of large numbers of cells. The color of the specimen was white, rather than grayish yellow and the cloud did not settle on standing. When first encountered I was surprised to receive reports of low counts from such opalescent fluid. In addition, in spite of a falling count, the opacity of the serum remained rather uniform. In two other cases, I noted this clouding in a slight degree, three days following the administration of serum. These two cases had no evidence of reaction except very severe headache.

I was led by the work of Delahet to investigate the precipitin formation in the spinal fluid of a case of meningococcal meningitis under treatment. A portion of the fluid removed prior to treatment each day was layered with horse serum (tetanus antitoxin) and placed in the incubater at 37.5°C. for eighteen hours. The fluid removed on the fourteenth puncture reacted with the horse serum at the point of contact, as evidenced by the formation of a clear cut white ring about 1 mm. in width. The patient had shown no evidence of anaphylactic reaction but serum therapy was discontinued at this point. The ring formation had entirely disappeared after two more punctures. The ring formation is independent of the protein content as fluids with a total protein as high as 75 mgm. have failed to react with the horse serum.

Manwaring, who worked with dogs sensitized to horse serum, washed the livers of these dogs free of blood by a preliminary perfusion of Locke's solution. They were then perfused with Locke's

solution containing horse serum. The perfusate which had previously been clear suddenly became milky.

In consideration of the above statements, I suggest as a working hypothesis that the gross cloudiness of our spinal fluids, the reaction of one fluid with horse serum and the cloudiness occurring in Manwaring's perfusates, represent a single process, namely, the formation of precipitins in the presence of an excess of precipitinogen (horse serum). I further suggest that the severe meningeal reactions observed by myself and others result from the irritation of the meningeal surfaces by the flocculent precipitate which is thrown down when the spinal fluid comes in contact with the injected horse serum. The precipitins may act merely as mechanical irritants to the meningeal surfaces or it is possible that there is some more specific type of reaction involving the mesothelial cells.

TREATMENT AND PROPHYLAXIS OF MENINGEAL REACTION

In the treatment of the reported case, large doses of epinephrine were used without apparent benefit. The intravenous injection of hypertonic solutions of glucose and repeated spinal drainages were equally ineffectual.

Delahet advises the injection intraspinally of small quantities (5 cc.) of the patient's inactivated blood serum.

It is manifestly important to establish, if possible, some reliable sign of impending meningeal reaction. It has been stated that a rising glucose value in the spinal fluid may be taken as an indication of probable reaction. I feel that this is, however, merely a component of the relatively harmless serum meningitis and bears no relation to the true anaphylactic reaction.

As a more reliable guide, I suggest the overlaying of each specimen of spinal fluid with an equal quantity of horse serum. If a ring appears, at the point of contact, serum therapy should be discontinued unless the indications for administration outweight the possible dangers of reaction. If serum therapy must be continued, the administration of old sera or serum inactivated at 56°C. for forty minutes is advised. The latter procedure is uniformly applied to all sera prepared by the Pasteur Institute and is

believed to account for the lower percentage of serum sickness in France (13 per cent). It is especially important to avoid the intravenous use of serum subsequent to intraspinal administration.

SUMMARY

1. An infection obtained in the laboratory with the meningococcus is reported.

2. A peculiar anaphylactic reaction occurring in the course of serum therapy, and apparently involving the nervous system alone, is described.

3. The meagre literature relating to the action of serum on the nervous system is reviewed.

4. Evidence is offered to indicate that the described reactions are due to irritation of the meninges by the formation of precipitins in the presence of an excess of precipitinogen (horse serum).

5. A method for the detection and prevention of impending meningeal reactions is described.

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THE PRESENT STATUS OF KNOWLEDGE OF CANCER*

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This great mechanical, scientific and publicity age, although it has done much for civilization, has nevertheless failed to eliminate superficiality of knowledge, and snap judgments from enthusiasm. These three factors are obvious in the mental attitude of members of the medical profession and laymen in dealing with the economic problem of cancer. It is natural for all to have an emotional attitude toward this disease which destroys so many men and women in the best and most productive period of life. Naturally we all wish to curtail and eradicate this activity. The problem is not a new one although there is much circumstantial evidence for believing that its importance is increasing.

Every five years for the last quarter of a century I have reviewed the literature in the hope of finding new observations which might be correlated for the purpose of evolving a practical approach to this problem. The abundant literature deals with frequency of the disease, its cause, natural and experimental immunity, the body's natural defensive mechanism, all kinds of diagnostic and prognostic methods and schemes, and many kinds of surgical, radiologic, serologic, dietary, purely physical and chemical methods of treatment. The sources of knowledge are special institutions for research, clinics, hospitals, and private practices all over the civilized world. The literature is too voluminous for any one man to review completely; even the professional abstracters lack completeness in their work.

Recently Woglom reviewed and summarized the experimental data on immunity; he concluded as follows:

"Immunity to transplantable tumors is a generalized refractory condition which appears to be entirely unrelated to other forms of immunity. No single organ has yet been proved responsible for its elaboration, nor is it affected by physiological conditions such as age or pregnancy. In its acquired form it is neither hereditary nor passively transferable through the body fluids. It seems probable that natural resistance is only the ability to react so promptly and efficiently that a graft is overcome before ever it gains a foothold. The outcome of inoculation is determined by an interplay between the hostility of the host and the proliferative vigour of the implant; hence an absolute immunity does not exist. Resistance is effective during the first few days following the inoculation, but entirely powerless against an established tumor. Nothing may accordingly be hoped for at present in respect to a successful therapy from this direction."

The lay press, in its attempts to present the truth of science, had done much to enlighten the public, to stimulate an interest in early diagnosis and to help eradicate this disease, as it has done with tuberculosis and as it is doing with disease of the heart, lungs, and kidneys. The publicity often has been spectacular and as such has been a great stimulus, although often misleading, especially to patients. Here and there new means of diagnosis and new cures are proclaimed only to recede and then fade into the same obscurity that has characterized panaceas that have appeared in the literature on cancer for centuries.

The medical profession should do more to encourage experiments, trials, and new methods regardless of where and by whom they are made. Professional discredit of newspaper cures may be just as immature and unscientific as the cures and methods themselves. In brief, I know of no specific cures for cancer but I do know of hundreds of patients who once had cancer which has been cured, if the prolongation of useful life beyond the average length of life is considered a cure.

In our enthusiasm for research we have often neglected the good that is being accomplished; we overlook the fact that cancers are being cured and probably prevented every day. I could enumerate many instances of patients who were treated for cancer ten, fifteen and twenty years ago and who are now useful citizens. Add these years to the average age of the patients at the time of treatment and the results are extremely good, considering what would have happened had they not been treated.

After having studied 32,792 patients with cancer I do not feel that the disease is hopeless. In fact I know that it is not hopeless when recognized early, any more than pulmonary tuberculosis, nephritis, cardiac disease, gun-shotwounds, fractures of the legs, hemorrhages and many other common diseases are hopeless. The handling of the cancer problem is quite simple. It rests with the medical profession, a profession which is characterized by traditional ultraconservatism. As long as the medical profession waits for characteristic diagnostic signs and symptoms of cancer just so long will cancer be the same problem it is today. Some of us no longer wait for emaciation, pulmonary hemorrhage, daily rise of temperature, and night sweats before we make the diagnosis of tuberculosis; neither should we wait for palpable masses, cachexia, anemia, lymphatic involvement and metastasis in cases of cancer before making the diagnosis and instituting treatment. The majority of patients with cancer can pass easily the usual insurance examination if they say nothing of symptoms related to cancer; their weight is normal, they are not anemic, their blood pressure is normal, their hearts are normal, they have no albumin, blood cells, or casts in the urine, and no palpable masses. Their condition would not be classified as fit examples of cancer for teaching students in our medical schools and neither would their condition answer the description of cancer in textbooks. Most general pathologists would not see such conditions because the patients have not been subjected to operation or necropsy. Persons with curable cancers are walking about the streets, and there are probably between 3,000,000 and 6,000,000 among us waiting for signs and symptoms before the diagnosis will be made by textbook methods. Figures 1, 2, 3 and 4 illustrate the size of cancers as we see them. It may be seen that it is quite possible to recognize small cancers. Then why is the average cancerous growth so large before radical treatment is instituted? It is of interest that 50 per cent of all cancers of the stomach observed in The Mayo Clinic are inoperable and hopeless, and only half of the remaining 50 per cent are small enough to be removed. Thus only 25 per cent of all patients with cancer of the stomach when seen in the clinic have any possible chance of
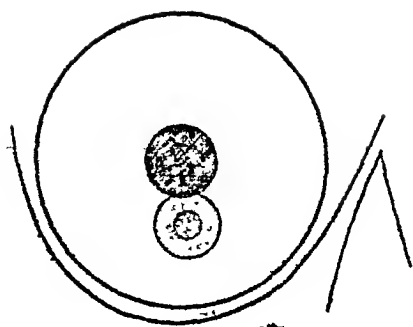
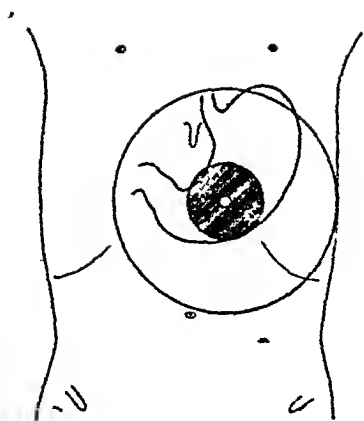FIG. 1   FIG. 2

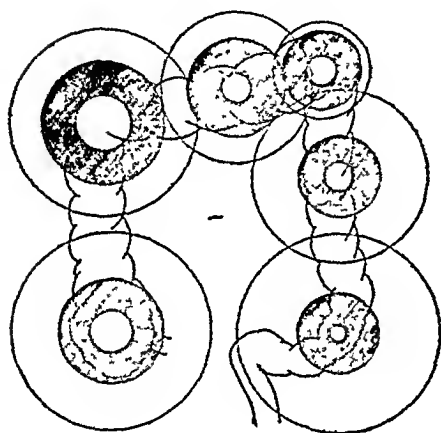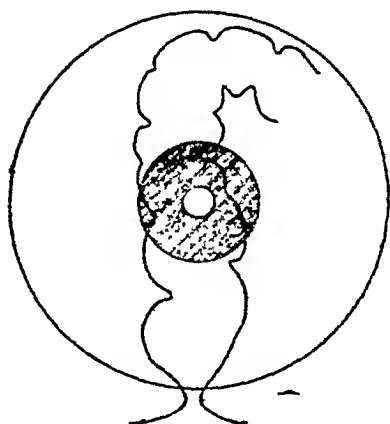
FIG. 3   FIG. 4

FIG. 1. DIAGRAM OF THE BREAST SHOWING THE LARGEST (LARGE CIRCLE), SMALLEST (SMALL CIRCLE), AND AVERAGE SIZE (SHADED CIRCLE) OF SURGICALLY REMOVED CANCERS

FIG. 2. DIAGRAM OF THE STOMACH SHOWING THE LARGEST (LARGE CIRCLE), SMALLEST (SMALL CIRCLE), AND AVERAGE SIZE (SHADED CIRCLE) OF SURGICALLY REMOVED CANCERS

FIG. 3. DIAGRAM OF COLON SHOWING THE LARGEST (LARGE CIRCLES), SMALLEST (SMALL CIRCLES) AND AVERAGE SIZE (SHADED CIRCLES) OF SURGICALLY REMOVED CANCERS IN THE CECUM, HEPATIC FLEXURE, TRANSVERSE COLON, SPLENIC FLEXURE, DESCENDING COLON AND SIGMOID (INCLUDING RECTOSIGMOID)

FIG. 4. DIAGRAM OF RECTUM SHOWING THE LARGEST (LARGE CIRCLE), SMALLEST (SMALL CIRCLE), AND AVERAGE SIZE (SHADED CIRCLE) OF SURGICALLY REMOVED CANCERS

relief and possible cure. The therapeutic results in this 25 per cent are not so bad, considering the late stage of the disease (table 1).

In a series of 200 resected gastric cancers which I studied especially for the relation to postoperative longevity of lymphatic involvement, cellular differentiation, lymphocytic infiltration, fibrosis and hyalinization, I found data in regard to lymphatic involvement as shown in table 2.

TABLE 1
Therapeutic Results

	Average size of lesion	Percentage of patients alive after more than						
		Three years	Five years	Six years	Eight years	Ten years	Eleven years	Fifteen years
	cm.							
Breast with lymphatic involvement.....	3.92	36.6	21.9		18.9			
		39.2	24.2			13.3		
Breast without lymphatic involvement..	2.53	75.6	65		63.9			
		74.6	63			44		
Breast regardless of lymphatic involvement...................	3.2	44.7					21	
Uterus...................		62.7					22	7.2
Right half of colon..................	8					32.3		
Stomach, 1899 to 1909.................	6.16	29						
Stomach, up to 1911..................	6.16		22					
Stomach, 1897 to 1917.................	6.16	38.6	26	14.6				
Stomach, 1897 to 1919.................	6.16	37.6	25					

Again I wish to emphasize the fact that these are very good results considering that in all of these cases clinical signs and symptoms of cancer were very obvious. They were not the early cancers which are being seen today. The next ten years will show better results because a greater number of small cancers without lymphatic involvement are now being discovered as a result of roentgenology and surgical exploration of conditions such as gastric ulcer, localized chronic mastitis, intestinal hemorrhages,

obscure anemia, and especially unrelieved abdominal distress without specific organic diagnosis.

In 6149 cases of cancer of the breast, stomach, and large intestine, lymphatic involvement was found in 61.2 per cent at operation. These patients were carefully selected for operation, many more than that number having been refused operation.

The point I wish particularly to emphasize is that it is not fair or economically wise to discredit the good results of surgery, radium, and roentgen ray while we are hoping for some specific serologic cure for cancer. If we as a profession forget these good results and lend publicity only to immature and possible but

TABLE 2

RELATION OF LONGEVITY TO LYMPHATIC INVOLVEMENT

LENGTH OF LIFE AFTER OPERATION, MORE THAN	CASES WITHOUT LYMPHATIC INVOLVEMENT	CASES WITH LYMPHATIC INVOLVEMENT
years	per cent	per cent
1	78.7	50
2	60	25
4	33	14.4
6	21	7.6
8	12	6.7
10	6	0
11	2	0
13	1	0

untried curative means, the layman might be led away from treatments which have already proved their value even under the least favorable circumstances. I wish to emphasize also that early recognizable cancer can be cured and every good surgeon and radiologist knows this. Their only gloom arises when they think of the enormous percentage of cancers which might have been cured had they been seen earlier and had not been held back by some one waiting for the signs and symptoms of cancer which never appear except in the late stages.

CONCLUSIONS

There is no openly demonstrated cure for cancer other than radical removal by operation, or the application of radium or

roentgen ray, or combinations of these. This is an open challenge to those who make other claims. If they accept the challenge and openly demonstrate their results to be better than those I have mentioned then we shall acknowledge gladly their great value.

We should encourage research and be open minded even in the face of doubt but we must do everything possible to keep the public free from false hopes. We should lend every effort to our newspaper friends in their attempts to give valuable scientific facts to the reading public; we should help them decide fairly just what is still experimental and should be kept within the confines of the laboratories until the truth has been substantially demonstrated before scientific bodies.

EDITORIAL

American Journal of Clinical Pathology

For several years, the Executive Committee of the American Society of Clinical Pathologists has studied the possibility of publishing an official journal for the Society. During this period many propositions have been considered, investigated and abandoned. Through an arrangement with The C. V. Mosby Company and Dr. Warren T. Vaughan, the Editor, the Society's notices and the papers presented at the annual convention have been published in the *Journal of Laboratory and Clinical Medicine*. Relations between this Journal and the Society have been both pleasant and profitable. The Society owes a debt of gratitude to the owners of the Journal.

However, as the Society grew it became evident to the Executive Committee that it would be more advantageous for the Society to own and control its own publication. Accordingly, largely through the efforts of Dr. John A. Kolmer, an arrangement has been entered into with The Williams & Wilkins Company, Publishers of Scientific Journals and Books, for the publication of a bi-monthly journal of about 450 pages per volume per year.

By careful management it will be possible to send the Journal to each member of the Society without an increase in dues. If additional subscriptions can be secured and if sufficient high-grade advertisements are obtained, the Journal can be increased in size.

Although the primary purpose of the Journal is to furnish a prompt outlet for the papers presented at the meetings of the Society and for other papers written by members of the Society, it is not proposed to limit publication to papers of members. Non-members of the Society may also submit manuscripts which will be accepted by the Editor if they meet the standards of the Editorial Board and if space is available.

The pages of the JOURNAL will be devoted to original articles dealing with clinical pathology as interpreted in its broadest sense. Contributions dealing with new methods, with comparison of old methods, and with applications of clinical pathology to medicine and surgery will be welcome, as will articles on bacteriology, chemistry, pharmacology and physiology as related to laboratory problems in medicine and surgery. Hence the scope of the JOURNAL will be commensurate with the field of clinical pathology.

In addition, the JOURNAL will publish editorials on current laboratory problems, summarizing and commenting upon important investigations. Lastly, the JOURNAL will present a summary of Society activities, as well as important and significant accomplishments of its members.

Elsewhere in this issue will be found certain rules and regulations relative to the form in which manuscripts are to be sent to the Editor. Coöperation in fulfilling these requirements will result in a saving of expense to the Society which will be reflected in an increase in the size and scope of the publication.

It is, of course, hoped that a large number of papers will be submitted in order that the highest type may be selected for publication in the JOURNAL. Naturally concise short articles are most desirable and in choosing and editing papers this will be kept in mind.

The Editor and the Advisory Editorial Board pray for the hearty support of the members of the Society in this new project and invite comments and criticisms that the JOURNAL may not only assume a position of importance among scientific periodicals but may become outstanding in its field.

T. B. M.

SOCIETY NEWS AND NOTICES

NINTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

June, 1930, Detroit, Michigan

A banquet was held in the Book-Cadillac Hotel in Detroit, Michigan, Saturday evening, June 21, 1930, at seven o'clock. Dr. J. H. Black read the presidential address entitled "Biology of Clinical Pathologists" which will be published in full. Dr. Black introduced Dr. M. T. MacEachern who spoke of the usual activities engaged in by his department of the American College of Surgeons in relation to clinical pathologists and surgeons. This was followed by Dr. N. P. Colwell from the Council of Medical Education.

The Ward Burdick award for the year was made to Dr. H. J. Corper. The awards for the scientific exhibits were made to the following: first award to Drs. E. R. Mugrage and Jones of Denver, Colorado; second award to Dr. T. J. Curphey of New York.

Meeting adjourned at ten o'clock.

Meeting of the executive session was held on Monday morning, June 23, 1930, at nine thirty o'clock. The meeting was called to order by Dr. J. H. Black. Reading of the minutes was dispensed with since they had been previously published. Reports of committees followed.

REPORT OF EXECUTIVE COMMITTEE

WARD BURDICK AWARD

In consideration of the manner of awarding the Ward Burdick medal which was referred to the executive committee at the 1929 annual session, the following action has been taken:

The secretary or chairman of the program committee is to require an abstract

with the title of each paper submitted for the program so that it may be determined whether the paper merits a place on the program or not, and in the second place so that the research committee may have abstracts of all papers on the program before the meeting, enabling them to form some idea of the character of the papers to be read and judged.

Determined that there shall be only one Ward Burdick medal awarded each year and that if there is more than one author, the several names shall be engraved on this medal.

The research committee shall reserve the right to withhold the award during any year in which a paper of sufficient merit is not submitted by the members.

HONORARY MEMBERS

The matter of honorary members has been considered by the executive committee at the suggestion of the president and it is the opinion of the committee that we should invite certain distinguished pathologists to become honorary members. The names of Dr. William H. Welch, Rear Admiral E. R. Stitt and Dr. Louis B. Wilson are mentioned for consideration of the society.

OFFICIAL JOURNAL

Since the two years trial of the society's arrangement with the Mosby Company ends with this session, the executive committee suggests that the matter of the official journal be considered by the publication committee at this time.

FINANCIAL AFFAIRS OF SOCIETY

Motion was made, seconded and carried that it is the sense of the executive committee that the annual expenses of the society are more than is good for it and in consequence the secretary be requested to draw up briefly several schedules as to possible curtailment of various activities with the expenditure of a smaller amount of money and present the same schedules to the incoming executive committee when these schedules are ready for their consideration.

Motion was made, seconded and carried that the stenographic report of the society be eliminated from both scientific and business sessions including the present meeting. This provision is a suggestion that will necessitate all committee reports to be made in writing.

The report of the secretary-treasurer as to the present accounts of the society is considered favorably after a review of the summarized report of a certified accountant.

Motion made, seconded and carried that the account of registry for technicians be carried as a separate account from that of the society as it is expected that the registry will carry its own expense.

F. W. HARTMAN,
Chairman.

Motion made and seconded for acceptance of above report. Carried.

REPORT OF EDITORIAL COMMITTEE

During the past year the papers submitted were as a whole of much higher quality than ever sent in before. There were a few which did not seem suitable for publication in their present form but a casual glance through the journal will show that almost all the papers presented were published. The papers were published in the January and February issues of the journal which is the fastest publication that these papers have ever had in the history of the society. On the other hand, the papers could have been published much sooner had they been received by the Editorial Committee earlier. The Chairman of the committee received the first shipment of papers on October 21, 1929, and at that time no discussions of the papers were included so that we had to wait more than a month after that before the discussions were in.

With almost no exception, discussions of papers read in meetings are of very little value for publication. It is my feeling that if these discussions are not published that men will discuss the papers more freely and it is needless to say that very few men can carry at their finger tips pertinent facts concerning a variety of subjects which are usually presented at such meetings. I should therefore like to make the following two recommendations:

1. That hereafter the discussions of these papers not be published.
2. That the papers be sent to the office of the chairman immediately after they are read.

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T. B. MAGATH,
Chairman.

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The above report was read by the secretary.

The recommendations included within the report were voted upon separately. A motion was made and seconded that in the future, papers presented at the annual meeting shall have prepared discussions and these shall be published with the paper. Informal discussions will be permitted and will be printed only on condition that the discussant requests his remarks be published but must make them in writing and submit them to the secretary the day of the meeting so that no delay will take place for the publication committee. Carried.

Motion was made and seconded for acceptance of report. Carried.

REPORT OF PUBLICATION COMMITTEE

The Publication Committee wishes to report that the book being presented under the auspices of the society is practically completed with the exception of the section under chemistry. The committee feels that in all probability it will be ready for publication by next year and requests that the president appoint a committee to go over the subject matter for acceptance.

J. A. KOLMER,
Chairman.

Motion made and seconded for acceptance of report. Carried.

Motion made and seconded that the publication committee in conjunction with the executive committee be given the broadest possible power in the negotiation for the publication of an official journal. Carried.

REPORT OF PUBLIC RELATIONS COMMITTEE

The problems that this committee has more especially to consider include the relations of this society with those other societies and associations which have in part to do with the function of the clinical pathologist. There are also to be considered our relations to public health, hospital, private and commercial laboratories and laboratory technicians.

Some of the members, as your official representatives, have during the past year attended meetings of the American College of Surgeons, American College of Physicians, American Public Health Association and the Council on Medical Education, Licensure and Hospitals.

There appears to be, officially, an endeavor to coöperate with us and aid in improving the status of the clinical pathologist. In certain localities the coöperation between clinical pathologists themselves and between clinical pathologists and the above named organizations is enviable. It is hoped that ultimately this coöperation will extend to all parts of the United States. However, these organizations do not always exactly understand our position. For example we offer the reply of the American Medical Association to the request sent as a result of a resolution passed by the society at the Portland meeting, that clinical pathologists be not solicited for advertisements in the American Medical Association as they should be considered under the same code of ethics as other practicing physicians. Dr. West writes, "that the judicial council of the American Medical Association does not regard it unethical for clinical pathologists to advertise." We believe that if we try to look at it from a broad view we will realize that individually we are practicing physicians, but collectively laboratories are institutions and in the same position with relation to the medical profession as private hospitals are to the public, and there seems to be no objection to hospitals placing advertisements and stating the names of their staffs. The question as to whether we are to consider ourselves from this standpoint or not remains for the society as a whole to decide.

PUBLIC HEALTH LABORATORIES

Our relations and attitude toward public health laboratories continue to be undecided. We, as a representative organization, should continue to oppose whole-heartedly the methods by which these laboratories are encroaching on private laboratory work. Although it may be only when we can get the backing of the general practitioner and various organizations that we will be able to accomplish anything, still we should continue asserting that State or municipal Boards of Health should not do work that lies outside of strictly preventative medicine and that no examinations should be made on any but those who cannot afford to pay. The State, laymen and practicing physicians apparently do not regard it in the light that we do and they cannot be driven. This situation may be improved by education. All that we can expect is a fair opportunity to get the physician who desires laboratory examinations to realize that it is to his credit to give a patient who can afford to pay for it, laboratory examinations and advice that are purchased and not furnished free. When a practitioner wants consultation on a pay patient, he does not expect nor does the consultant expect to furnish it free. He does not call in a consultant who specializes in the care of free patients, but one who has made a name for himself and has a right to command a commensurate fee. So it should be with clinical pathologists.

HOSPITAL LABORATORIES

More and more hospitals are improving in the laboratory service furnished. There is a continuing demand for clinical pathologists to take control of hospital laboratory services. In most places clinical pathologists have some connections with one or more hospitals and the main demand is for more clinical pathologists to direct hospital laboratories. Unfortunately, in some hospitals, technicians are substituted with some member of the clinical staff figuring as head of the laboratory. In the smaller cities and towns which cannot support private laboratories, hospital laboratories necessarily are called upon to do outside work. In the larger cities where there are and rightfully should be private laboratories, we should be opposed to hospital laboratories doing outside work and especially at hospital prices on the ground that hospitals as institutions are competing with us as physicians practicing our specialty. Further, in doing this the hospitals are commercializing the services of their clinical pathologist by competing unfairly with clinical pathologists who operate private laboratories. Those hospitals doing outside laboratory work should be as carefully checked and inspected as outside private laboratories. The system of inspection in effect in New York State should be adopted by all States.

Here we wish to bring up an important point in regard to the comparison of fees that are charged by hospital and private laboratories. Through the county medical societies in any community the laboratory fees should be so fixed that the hospital should have no right to underbid a private laboratory for

laboratory services.   This is a growing evil and a menace to the clinical pathologists who hold hospital positions as well as those engaged only in private work. The fact is that some hospitals are reversing their age-old status as an adjunct to the physician in treating the illnesses of mankind to that of commercial institutions in active competition with more than one specialty in medicine.   In many hospitals that may otherwise be considered ethical, there is a deplorable cutting of laboratory rates which is unfair to outside competition.   We have no complaint to make of pauper patients, but of the pauperizing of patients by public health and hospital laboratories.

We have had several complaints in regard to this.   It is the opinion of the committee that steps should be taken by the society so that certain minimum fees may be adopted for laboratory services.   We cannot, however, recommend a definite fee that would suit all parts of this country but minimum fees can be recommended and steps be taken to bring them to the attention of all concerned.

It is the opinion of the committee that in addition to all the private laboratories, hospital and public health laboratories should have a proper rating as to their efficiency and standing.   We have gone on record as approving and offering our coöperation to the American Medical Association in the classification of laboratories.   Like all such beginning undertakings as in classifying medical schools and hospitals, there are bound to be mistakes made.   We may be assured that before long this classification will bear excellent results; just as it has done in the raising of a standard of medical schools and hospitals.   If the attempt at hospital standardization had not made the progress it has, the demand for clinical pathologists would not be as great as it is now.

We should also be in touch with the Committee on the Cost of Medical Care, to present our views in regard to laboratory cost for the patient, and the income derived by the clinical pathologist from his profession.

LABORATORY TECHNICIANS

We should realize that competent laboratory technicians are as essential to clinical pathologists as nurses are to a hospital.   Laboratory technicians should only be employed under the supervision of one competent to direct and check up on every detail of their work.   If nurses took over the care of the sick without being under the direction of a physician, the practicing physician would loudly complain.   In many cases technicians are employed by those who are incompetent to direct and supervise the accuracy of their work.   Therefore, we should condemn the employment of technicians in laboratories of physicians, commercial or hospital laboratories where they are not under the supervision of a clinical pathologist.   This could be done through the control of technician registration.

F. B. JOHNSON,
Chairman.

(Report read by Dr. B. W. Rhamy.)
Motion made and seconded for acceptance of report. Carried.

REPORT OF SERVICE BUREAU

The Service Bureau of the American Society of Clinical Pathologists was organized for the purpose of bringing in touch those members of the society seeking a change in location and institutions desirous of securing the services of competent men of this specialty to direct their clinical laboratories.

The Service Bureau Committee reports progress in its activities. It has received communications requesting aid in obtaining clinical pathologists and has notified members seeking new surroundings of these various opportunities. It is gradually becoming an important and necessary function of the American Society of Clinical Pathologists.

H. J. CORPER,
Chairman.

Motion made and seconded for acceptance of report. Carried.

REPORT OF RESEARCH COMMITTEE

Your Research Committee wishes to report a considerable progress in combined work, in that this year fifty-nine questionnaires were returned with details on many extremely interesting cases. The summary of these cases was taken up in discussion at the Symposium on Agranulocytosis and will be published in full in the JOURNAL. The subject was chosen in order that by thorough discussion of the problem a better understanding of exceptional hematological cases might be obtained. Many of the cases reported were lacking in features which were essential to a proper diagnosis. By a thorough threshing out of this interesting subject, it is expected that our own membership will be more able to handle such cases in the future.

It is interesting to note that in the questionnaires returned, 137 cases of undulant fever had been diagnosed before July, 1929, and by the same men from 1929 to 1930, 111 cases. The agglutinin titer of the blood in these cases averaged 1:320 and 1:640, with some higher and only one man reporting lower values. Blood cultures have been positive in eleven cases, one man reporting 103 cases for both years had seven positives and four other scattered cases were positive out of the total series of 248 reported. This corresponds very well with the similar studies conducted by the New York State Association of Public Health Laboratories in which less than 5 per cent of the cases yielded positive blood cultures.

Apparently our membership had little inclination to try to duplicate L'esperance's work on Hodgkins' disease because only one member reported injection of chicken and this experiment was inconclusive. Your chairman can report two entirely negative cases and can quote also six negatives by Esmond Long of the University of Chicago.

In regard to the Ward Burdick Medal, we regret that no papers were received for perusal by the committee by the appointed date. Apparently the method we chose for selection of the winner was not the proper one. However, your committee does not feel discouraged in the matter since many other societies have the same problem. We hope that a modified plan made at the meeting of the executive committee will settle the problem for future years.

Some members of the committee feel that it would be a good plan to consider problems for joint study a few years in advance, so that definite plans can be drawn up for all the members to follow and material from the various parts of the county will be comparable. Such a plan particularly is apropos in the study of vaccines and vaccine therapy as suggested by Dr. Black. This work we feel should be carried on for more than a year; particularly new cases should be considered following a certain set plan and specifications. This is being done by the committee on vaccines.

We suggest that the subject of agranulocytosis or blood conditions associated with marked leukopenia be studied during the next year, and forms regarding details necessary for complete study of cases be sent to the members as soon as possible if this subject is chosen.

We thank the President and Secretary for their most earnest coöperation and help, and also the other members who have reported their cases or given help in other ways.

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A. G. Foord,
Chairman.

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Motion made and seconded for acceptance of report.   Carried.

REPORT OF SCIENTIFIC EXHIBITS COMMITTEE

The Scientific Exhibits Committee made a distinct effort throughout the year to obtain as large a number of exhibitors as possible. This was accomplished by circular letters to the membership at large and by personal appeal. We were discouraged however, the net result being two additional exhibitors to the original voluntary exhibitors.

We believe that the offer of awards by the executive committee was useful in stimulating good work. However, the task of choosing the winners was decidedly difficult. The first award went to Drs. E. R. Mugrage and Jones of Denver, Colorado and the second award to Dr. T. J. Curphey of New York, New York.

We thank the officers for their coöperation in our work.

We believe that the obtaining of scientific exhibits should be given sincere attention, and that the question of scientific exibits should be a major part of every convention.

There are no recommendations to be offered.

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C. I. Owen,
Chairman.

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Motion made and seconded for acceptance of report. Carried.

REPORT OF NECROLOGY COMMITTEE

Whereas, we have lost by death two of our members, Dr. George D. Fussell and Dr. Annymarea P. Saunders, be it resolved that we express our sincere sympathy to the families of these two departed colleagues, and that the obituaries of Dr. Fussell and Dr. Saunders be published in the official journal of the American Society of Clinical Pathologists.

OBITUARIES

Dr. George D. Fussell died at the Clearfield, Pennsylvania Hospital, November 30, 1929 after a brief illness at the age of forty-one years. He was the son of Dr. M. Howard Fussell, Professor of Applied Therapeutics at the University of Pennsylvania Medical School. Dr. George Fussell became associated with the Clearfield Hospital in 1921 and achieved an enviable reputation as a clinical pathologist.

Dr. Annymarea Petersen Saunders died of pneumonia, January 1, 1930. She was born in 1888, graduated from the University of Iowa, College of Medicine in 1912, and licensed to practice medicine in Illinois in 1915. She was ably prepared to practice her specialty, and was formerly in charge of the clinical laboratories and x-ray laboratories at the University Hospital, Chicago, Illinois. From 1921 until the time of her death she was employed as clinical pathologist for the State Psychopathic Institute at Chicago, Illinois.

A. H. SANFORD,
Chairman.

Motion made and seconded for acceptance of report. Carried.

AGRANULOCYTOSIS: RESEARCH COMMITTEE REPORT

A questionnaire was sent all members of the American Society of Clinical Pathologists in 1930, in order to collect information about the frequency of and details about cases of agranulocytosis angina and related blood dyscrasias. Sixty cases were reported, exclusive of those presented by Drs. Rosenthal and Miloslavich at the Detroit meeting. None of the reports were obtained from the larger medical centers. In classifying the cases considerable difficulty was encountered, since full details were not obtainable by any practical type of questionnaire. However, of the entire group thirty-eight can be fairly justly classified as showing agranulocytosis, and a clinical and laboratory syndrome of the Schultz type. The summary is best seen in the tabulation.

Of the thirty-eight cases, eighteen were in males and twenty in females. The ages varied from seventeen to seventy-three years, the average being forty-two.

Collected cases of agranulocytosis

NUMBER	SEX	AGE	Gums	Throat	Anus	TEMPERATURE	DURATION (days)	OUTCOME	ARSPHENAMIN TREATMENT	JAUNDICE	HEMOGLOBIN per cent	ERYTHROCYTES	LEUCOCYTES	POLYMORPHONUCLEARS per cent	MONOCYTES per cent	LYMPHOCYTES per cent	PLATELETS	BLOOD CULTURE	AUTOPSY	MYELOCYTES IN MARROW	REMARKS
1	M.	30		Cheek		103 103	7	Died			63	3.0	1,000	0		91		—			Transfusions and salvarsan. Improved
2	M.	68		+		101	8	Died			70	4.0	100 450	0 0		100		—	+	Not examined	Absence of polymorphonuclears in tissues
3	F.	50	+	+		105	11	Died			51	2.8	800	1		99	Normal	—	+		Red femur marrow—polymorphonuclears on last day
4	F.	53	+	+		105	11	Died		Slight	53	3.5	800 2,500	0 80	5	15	Normal	—	+		Studied two hours before death only
5	F.	49	+	+		102 103	0	Died			61	3.5	700	0		100		Diplococcus pneumoniae	+	+	†Died of pneumonia 2 months after recovery from agranulocytosis. Polymorphonuclears and myelocytes in bone marrow
6	F.	61	+	+	+	102	90	Living†			80	3.8	1,360 3,200	4 0	21 7	70 77	120,500		+		
7	M.	25			+		90	Living					2,100 2,800 7,400	17 2 83	0	79 81 17			Living		Recovered
8	F.	53	+	+		100 101	12	Died			73	4.4	600	0		100		—	+		
9	M.	73		+			7	Died		Slight	62	4.2	890	5		95		—	+		Many ulcers in stomach and gut

Rotated tabular data (cases 10–22). Values are transcribed to the best possible reading; many cells are faint.

No.	Sex	Age	A	B	Temp (°F)	Days	Result	C	D	col I	col II	Count	col IV	col V	col VI	Normal	Bacteria	Outcome	Remarks
10	M.	52	+		101 / 210	210	Died	+			4.5	2,800	0	17	53		Strep. pyogenes	+	Two remissions to normal
11	F.	35	+		101 / 100	5	Died		Slight	75	4.0	200	3		07		—		Terminal pneumonia with absence of polymorphonuclears
12	M.	26	+	+	101 / 105	8	Died			100	5.2	1,100	0	16	81 / 87		—		
13	F.	24		+ (Check)	103	4	Died	+				1,350	0	0	100		—		Had counts of 8,000 to 12,000 with 60 to 70 per cent polymorphonuclears six months previous
14	M.	56	+	+	101 / 104	5	Died				3.0	1,400	2 / 122	41	51		—		
15	F.	20	+		102 / 103	10	Died					350	1	7	91		Staph. albus		
16	M.	52	+	+	105	3	Died	+		93		850	0.5	2	91.5	Normal	—		Died of pneumonia
17	F.	53	+		100 / 105	3	Died			61 / 50	3.4 / 3.7	1,100 / 400	0 / 0	8 / 3	92 / 97		—		
18	M.	73	+	+	101	29	Died		Slight	78 / 101	4.4 / 5.8	2,300 / 24,800	1 / 89	56 / 22	00 / 13		—		Agranulocytosis followed by terminal infection and leukocytosis
19	F.	20	+		102 / 105	18	Died			50	3.5	1,100 / 600	2 / 0	10 / 0	42 / 73		—		Followed phatic on palate
20	M.	55	+	+	101	4	Died					1,050 / 600	15 / 10	11 / 3	75 / 81		—		Followed arphenamine and mercury for syphilis
21	F.	52	+	+	103	12	Living			78	4.0	1,600 / 6,300	2 / 61	8	87 / 36		—	Living	Treated with transfusions and salvarsan
22	M.	21	+		101 / 103	21	Living			55	2.0	1,100 / 2,700	3 / 8	13	83 / 70		—	Living	Recovery in twenty-one days

Collected cases of agranulocytosis—continued

NUMBER	SEX	AGE	LESIONS			TEMPERATURE	DURATION (days)	OUTCOME	ARSPHENAMIN TREATMENT	JAUNDICE	HEMOGLOBIN (per cent)	ERYTHROCYTES	LEUCOCYTES	POLYMORPHONUCLEARS (per cent)	MONOCYTES (per cent)	LYMPHOCYTES (per cent)	PLATELETS	BLOOD CULTURE	AUTOPSY	MYELOCYTES IN MARROW	REMARKS
			Gums	Throat	Anus																
23	F.	29	+	+		102 / 103	8	Died			60 / 40	3.2 / 2.9	800 / 700	1 / 0	8 / 5	90 / 95		—			
24	M.	57	T				60	Living					2,800 / 7,500	8 / 61				—	Living		Abscess of jaw. Treated by transfusion and typhoid vaccine. Well thirteen months later
25	F.	20		+		98 / 105	100	Died			80 / 80	4.1	950 / 10,500	0 / 92	3	100 / 0		—	+		Several remissions. Death from pneumonia, with leukocytosis
26	F.	55		+	+	98 / 105	10	Died					700 / 500	1 / 0		90 / 100		—			Marked involvement of throat
27	F.	30	+	+		100	7	Living			85 / 81	1.1 / 4.7	1,600 / 4,200	4 / 17	15 / 1	91 / 52		—	Living		Infection of tongue following needle prick with recovery in seven days
28	F.	33	+	+		101	10	Living			60	3.2	2,800	13	1	87		—	Living		Infection in month following teeth extraction. Recovery
29	M.	23	+	+		102	60	Living	?		85	4.8	1,000	20	0	62		—	Living		Arsphenamin six months before onset. Later recovery after transfusion
30	M.	22	+	+		103	4	Died	+		80 / 80	4.2 / 4.8	3,200 / 1,800	3 / 12	5 / 7	92 / 81		—			Sudden onset after one of several arsphenamin injections
31	F.	34	+			101 / 103	42	Living			69 / 89	3.9 / 4.0	1,050 / 5,750	8 / 61	13 / 3	74 / 33		—	Living		Recovery in six weeks

																			Remarks
32	M.	47	+	+	+	102 / 101	8 Died	+	Slight	52 / 50	3.4 / 3.0	1,800 / 1,800	2 / 3	13 / 67	55 / 31	50,000 / 60,000			Throat involvement followed second arsphenamin. Active secondary lues
33	M.	30	+	+	+	102 / 101	18 Died			80 / 80	1.5 / 4.4	3,100 / 3,400	3 / 0	6 / 2	90 / 93	Normal		—	Sister died of aplastic anemia one year later
34	M.	30	+	+	+	102 / 101	8 Died	+		60	4.8	600	0	0	100	Normal		—	+	Disease developed in course of arsphenamin treatment
35	F.	55	+	+	+	103	4 Died	+		95	1.4	800	0	2	98	Normal		—	+	Died in relapse, following apparent recovery for two weeks
36	M.	21	+	+	+	103	4 Died			78	4.1	600 / 300	1 / 0	1 / 1	98 / 99	Normal		—	+	Typical onset during sulpharsphenamine treatment
37	F.	50	+	+	+	102 / 101	11 Died			85	4.7	2,000 / 1,800	0	0	100	Normal	Strep. (non-hemolytic)	+	Typical case
38	F.	60	+	+	+	102 { 102 / 101	11 Died			88	1.7	2,030 / 1,500	0	0	100	211,000	Strep. (non-hemolytic)	+	

* Disease followed treatment.

The gums or throat, or both, were involved in all cases, and a few showed ul-cerative lesions about the anus. High temperature, 102° to 104°, was the rule. Nine patients recovered. The duration of the disease varied from three days to eight months, (the latter in a case showing two remissions to normal), the average duration in the fatal cases without remissions being about seven days. Five patients developed the disease following some type of arsphenamine treatment for syphilis, one showing an extremely high monocyte count, similar to cases reported by others. Jaundice was present in only five cases, and in these slight icterus only. The hemoglobin and erythrocyte count was reduced slightly in all cases, but cases with profound anemia were not included. The average leukocyte count was about 1,000 to 1,200, one as low as 350 and one as high as 3,400. The polymorphonuclear leukocytes in seventeen cases, at one examination at least were absent, but on the average a few of these cells were seen. The monocytes varied greatly, lymphocytes forming nearly the entire number of leucocytes in most cases. Reports on blood platelets were made in only ten cases, but undoubtedly most of the cases showed no noteworthy changes. None of the patients showed purpura. Blood cultures were taken in thirty-three cases, and were negative in twenty-eight and positive in five. Bacteriological studies of the mouth lesions showed no constant flora. Autopsies were done on only eleven of the patients dying from agranulocytosis and in these myelocytes were absent or nearly completely so in all of the eight cases reporting bone marrow findings. An occasional case showed a hematologic return to normal and death soon after from an infection associated with an ordinary leukocytic response. In one case in a laboratory worker previous blood counts within a year of the onset of the fatal illness had shown normal findings. One developed a typical agranulocytosis blood picture accompanying an infection of the tongue following a needle prick, one followed removal of teeth and one followed a plastic operation on the palate. In all the autopsied cases lack or absence of granulocytes in infected areas was a striking feature.

For the coming year, 1930–1931, the committee intends to establish a registry for cases of agranulocytosis, purpura thrombocytopenia, and acute leukemia. The members of the society are urged to send in case histories, blood slides, and sections of biopsy and autopsy material. When sufficient material is on hand, it is to be used as a loan collection, the details of management to be arranged later.

Research Committee:
    A. G. FOORD, *Chairman,*
    A. H. SANFORD,
    F. E. SONDERN,
    W. M. SIMPSON,
    W. T. CUMMINS.

REPORT OF BOARD OF CENSORS

The following have been approved by the Board of Censors for membership in the American Society of Clinical Pathologists:

William C. Black
Emil Bogen
Rigney D'Aunoy
Norbert Enzer
Joseph W. Jackson
Howard M. Jamieson

Foster M. Johns
Gay B. Kim
Margaret M. Loder
Walter B. Martin (associate)
Bernhard Steinberg
Douglas R. Venable

Three names were held over for reconsideration during the next year.
Seven names were rejected.

The executive committee submitted the names of Rear Admiral E. R. Stitt, Dr. William Welch and Dr. L. B. Wilson for honorary membership in the society and they have been approved.

F. H. LAMB,
Chairman.

Motion made and seconded that the report be accepted and that those approved be admitted to the society. Carried.

AMENDMENTS TO THE CONSTITUTION AND BY-LAWS

CONSTITUTION

ARTICLE V

Section 5 (addition):
Suspension or expulsion from membership in the Society shall be by three-fourths vote of those members present and voting at a regular executive session.

BY-LAWS

ARTICLE VIII

Section 2 (change):
It shall be deemed unethical for members to publish objectionable laboratory advertisements in any form whatsoever. The Board of Censors to act as judges in the matter, the members having privilege of appeal to the Society at a regular executive session.

Section 3 (change):
It shall be considered unethical for a member to lend his name for publication in any laboratory advertisement or announcement, which violates the Code of Ethics. The borrowing of names of other physicians, scientists or laymen, on the basis of an occasional service or consultation, for purposes of advertising or to sanction the work of a laboratory is misleading and unethical.

Section 4 (addition):

Any system of secretly dividing or rebating fees for laboratory services shall be considered unethical.

Motion made and seconded for the adoption of the above amendments. Carried.

ELECTION OF OFFICERS

The nominating committee presented the names of the following:

President-elect: H. J. CORPER
Vice-President: C. I. OWEN
Secretary-Treasurer: A. S. GIORDANO

The candidates presented by the nominating committee were accepted unanimously.

Dr. Giordano then presented his resignation from the executive committee since the secretary-treasurer is or should be an ex-officio member of the executive committee, and he felt that this vacancy should be filled by another member. Resignation accepted.

The other names presented by the committee were:

Executive Committee

J. H. BLACK... 3 years
C. E. RODERICK.. 3 years
F. E. SONDERN... 2 years

Board of Censors

W. E. KING   C. W. MAYNARD

Board of Registry of Technicians

R. W. HAMMACK.. 3 years
W. G. EXTON.. 3 years

The above candidates were accepted unanimously.
The members retiring from the committees were:

Executive Committee: W. G. EXTON and A. S. GIORDANO
Board of Censors: R. OTTENBERG and C. H. MANLOVE
Board of Registry of Technicians: W. THALHIMER and E. S. MAXWELL

Note: Since the above elections Dr. Exton has requested that he be replaced by some other member on the Board of Registry of Technicians. President Lynch has chosen Dr. M. W. Lyon, Jr., to fill the vacancy.

REPORT OF THE BOARD OF REGISTRY

It gives me pleasure to report to you that the Registry of Technicians during the past year has gone forward with considerable success. Over four hundred laboratory technicians have been given certificates from our Registry.

It is gratifying to note the numerous letters of appreciation and comments that we receive from the licentiates who highly prize this testimonial of their qualifications and feel that their status is thereby greatly elevated.

The members of the Board of Registry are grateful to the colleagues in our Society who have been instrumental in calling the work of the Registry to the attention of their laboratory personnel.

The importance of the technician in the general hospital scheme and care of the sick is now universally recognized. At the last meeting of the Council of Medical Education and Hospitals, held under the auspices of the American Medical Association, in February, the subject of technicians was discussed by members of the Board of Registry.

The progress of the Registry of Technicians has been greatly strengthened by the friendly cooperation and encouragement of the American College of Surgeons. Dr. Malcolm T. MacEachern and his aides have been pointing out to the hospitals they visit the desirability of registering their laboratory technicians under the standards of the American Society of Clinical Pathologists.

The Registry has in quite a number of instances been of service to the members who have availed themselves of our Placement Bureau and obtained competent technicians on short notice.

One of the functions of the Registry is the supervision of schools for technicians. The work this year has been confined to collecting data from the application blanks that were sent out to various medical schools and universities having a course for technicians. We are not yet prepared to give the results of the survey of schools.

Judging by the progress made by the Registry in the two years of its existence, we may confidently predict for it a permanence and stability which will prove of benefit to the technicians as well as to the clinical pathologists.

Board of Registry
PHILIP HILLKOWITZ, *Chairman.*
KANO IKEDA,
ALVIN G. FOORD,
C. Y. WHITE,
E. S. MAXWELL,
WILLIAM THALHIMER.

EXECUTIVE COMMITTEE MEETING

The meeting of the executive committee was held in the Book-Cadillac Hotel, Monday afternoon, June 23, 1930, at one o'clock.

Dr. J. H. Black was appointed chairman of the executive committee by President K. M. Lynch.

Dr. A. H. Sanford brought up the question of publication of the journal. Dr. J. A. Kolmer suggested that he be permitted to negotiate with the Williams and Wilkins Company in regard to the journal. This was moved and approved.

Meeting adjourned.

THE HORMONE TEST FOR PREGNANCY*

HARRY L. REINHART and ERNEST SCOTT

Department of Pathology, Ohio State University

The function of the ductless glands may be investigated by two principal methods. The first method may be illustrated by the effects of the loss of the gland by disease, experimental removal, or extirpation, in which case the resulting symptoms are assumed to be the result of a hypoactivity of the gland, or of its absence. The second method consists essentially in the introduction of the gland, or of its extracts or secretions into the body of an experimental animal in order to produce reactions, which may be comparable to those brought about by an exaggeration of the glandular activity.

The function of the secretion of the anterior lobe of the pituitary body has been investigated by both of these methods. However, it is only in recent years, that the technique of the experimental investigation has been sufficiently perfected to allow one to make any very definite conclusions in regard to the functions of the gland. Besides the difficulty of the technical procedures, the problem has also been complicated by the effect of the hormone of the anterior hypophysis on other glands, especially the gonads, whose modes of functioning were also incompletely understood. Furthermore it would now seem that there is not one hormone secreted by the anterior lobe of the hypophysis, but three or possibly four.

As far as we have been able to ascertain, Horsley[14] was the first to publish regarding the experimental removal of the pituitary gland. He stated that he had removed the pituitary body from two dogs which were sacrificed at the end of five and six months respectively and that hypophysectomy led to no disturb-

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* Read before the Ninth Annual Convention of the American Society of Clinical Pathologists, Detroit, Michigan, June 20–23, 1930.

ing symptoms.   A truly remarkable statement when we consider the number of investigators in the next twenty years who were unable to maintain life in hypophysectomized animals for a sufficiently long time to observe any symptoms of importance. In fact, the question then arose as to the necessity of the gland for the maintenance of life.   Investigators from the time of Horsley until 1908 met with varying results.

Paulesco[15] successfully evolved a technique, which was a decided advance over any which had been previously used, and with this technique he obtained a long series of hypophysectomies. Of these twenty-four proved to be total, the average duration of life of all cases was twenty-four hours.   From a second grouping of seven partial hypophysectomies, he concluded that removal of the anterior lobe like total removal of the gland resulted in death of the animals, and that loss of the posterior lobe led to no appreciable disturbance.   Thus the hypophysis was concluded to be an organ indispensable to life.

Crowe, Cushing and Homans[4] conducted an extensive study of experimental hypophysectomy, from which they concluded that "total removal of the hypophysis leads inevitably to the death of the animal with a peculiar and characteristic train of symptoms which have been called cachexia hypophyseopriva." Death did not necessarily occur as rapidly as Paulesco claimed as the "puppies may remain in an apparently normal condition for at least three weeks before terminal phenomena appear."

Removal of the posterior lobe leads to none of the manifestations of cachexia hypophyseopriva.   Removal of the anterior lobe alone produces these symptoms.

The most striking feature of removal of the anterior lobe is a state of adiposity accompanied by a secondary hypoplasia of the organs of generation in adults or by a persistency of sexual infantilism in case the primary hypophyseal deficiency antedates adolescence.

Recent experimental studies on rats, cats, and dogs have shown conclusively that the hypophysis is not essential to life in these animals, and that although their span of life after hypophysectomy may be shorter than that of the control, it is of sufficient

length to indicate that the removal of the pituitary has not resulted in death.

Aschner[2] published striking photographs of hypophysectomized puppies of various ages which had remained infantile in appearance and size until sacrificed. From which we may conclude that (1) the loss of the anterior lobe of the hypophysis is not incompatible with life; (2) removal of the anterior lobe of the hypophysis brings about a train of symptoms, the most important of which, so far as we are concerned at this time, is failure of infantile sex organs to develop, and atrophy of adult sex organs.

These symptoms bear a marked resemblance to Fröhlich's[11] syndrome, which is supposed to be due to hypopituitarism, and is characterized by infantile genital organs and sex characters, and an associated disturbance of fat metabolism in which there occurs a marked and rather characteristic deposition of fat. From these observations we may conclude that there is a definite interrelation between the anterior lobe of the hypophysis, and the sex organs.

Having obtained a more or less definite train of symptoms as the result of extirpation of the anterior lobe of the hypophysis, both in experimental animals and as the result of disease in man, the next logical step was that of substitution therapy. Oral administration was instituted by Wulzen[21,22] and Goetsch[12] with contradictory results. Later workers, especially Evans and Long[6] and Smith,[18] have demonstrated that oral administration has no effect on the date of maturity, at least of rats.

Evans and Long[6] prepared an extract of bovine hypophyseal substance which they injected into the peritoneum of adult white rats and succeeded in producing very definite changes in the reproductive organs.

The results of these injections were as follows:

1. Estrus was absent or inhibited at long intervals.

2. The uterus remained infantile, but the ovaries were twice the size of those of the control animals.

3. Histological examination showed the presence of very abundant lutein tissue in the ovaries and the formation of this tissue about the egg in unruptured normal follicles and in atretic follicles. Ripe normal Graafian follicles were

invariably absent.   A powerful, specific stimulus to lutein cell transformation was thus present in this hormone.

These are the first definite results obtained from the injection of an extract.

While numbers of attempts had been made to observe the changes produced by whole living gland transplants, the results were unsatisfactory until Smith[17,18] using daily transplants of anterior lobe succeeded in overcoming to a large degree the effects of hypophysectomy by restoring an almost normal growth rate. Thus, knowing the symptoms produced by ablation of the anterior lobe of the pituitary, he was able to check the effect of his transplants by observation of the total, or partial absence, of the characteristic symptoms of hypophysectomized animals.   Having proved the effectiveness of such substitution therapy, he extended his observations to the results obtained by superimposing the transplants upon normal animals in various stages of development, thereby inducing a state of hyperstimulation.

Smith's work consisted in a series of daily anterior pituitary transplants, from mammalian donors, into immature female mice or rats.   Such transplants induced with striking rapidity a series of changes involving all parts of the genital system.   The vaginal introitus became established and permitted the insertion of a small spatula.   The vaginal smear indicated an oestral condition and that ovulation would soon take place.   If autopsy was performed, the uterus was greatly distended and hyperemic. Many follicles nearby, ready to rupture were seen on the surface of the ovary.

If autopsy was postponed for a day, there had been progress of the sex cycle, and the picture changed.   The uterus was not distended, but rugose and muscular.   The ovary contained numerous corpora lutea which had increased the size of the organ considerably above that of the pre-ovulation stage, and the vaginal smear revealed a post-oestral condition.   The changes in general then may be summarized as the inducation of precocious sexual maturity.

The most marked change was the greater size of the ovaries of animals, which had received the pituitary transplants in compari-

son to those found in control mates, which had received no transplants, but had matured normally. On the basis of relative percentage weights, the combined ovarian weights of treated animals were respectively 8, 9, 10, and 14 times that of untreated, or normally maturing controls. Thus in addition to producing a precocious sexual maturity, with pituitary gland transplants, an ovarian giganticism was produced. The enlargement of the ovaries was not due to any increase in size of follicles or corpora, but to an increase in number. Structurally no deviation from the normal was seen in either follicles or corpora.

Smith summarized these changes as follows:

The presence in the ovaries of the treated animals of a large number of approximately mature follicles or corpora, together with the relative absence of atretic follicles is correlated with the occurrence of very large numbers of tubal ova, found when autopsy is performed after ovulation. . . . . The number of these ova exceeds very considerably the greatest number ever reported for these species.

These ova cannot be distinguished histologically from any normal ova. These changes which occur in the ovary constitute the anatomical picture of superovulation.

Changes in other organs may be briefly summarized as follows: The uterus shows a slight increase in weight, as compared with normally maturing controls, while histologically the two are identical. The only marked change therefore, is a precocious, but otherwise normal uterine maturity. Vaginal changes consist in establishment of precocious introitus and oestrus.

The significant fact in regard to the uterine and vaginal changes is that following total ovariectomy, pituitary transplants have no effect upon the uterus or vagina.

If, however, the ovarian hormone (follicular) alone is administered to these spayed animals, the changes noted above occur. Furthermore following ovariectomy pituitary transplants will not prevent the atrophy of uterus and vagina which normally occurs with castration.[19]

These observations indicate that at least one action of the hormone of the anterior lobe of the pituitary body is directly upon the ovary, and that the changes in the ovary vary according

to the age of the recipient, inducing in the immature mouse or rat precocious ovulation, and in the adult, superovulation. Such changes in the ovary as a result of increased amounts of the pituitary hormone have led to the designation of the pituitary by Zondek as the "motor of the ovary."

Not only does the anterior pituitary lobe exercise a direct control over the development and activity of the gonads, but it in turn is influenced by the genital organs. Comte[3] and later others, have noted the hypertrophy of the hypophysis during pregnancy. Erdheim and Stumme[5] demonstrated that a cell type characteristic of pregnancy arises from the chief or chromophobe cells.

The hypertrophy of the anterior hypophysis following total extirpation of the ovaries was first found in animals by Fichera[8] and has since been repeatedly corroborated. Tandler and Gross[20] demonstrated an enlargement of the sella turcica of eunuchs by means of x-ray, and Rossle[16] has shown that castration in humans results in a gross hypertrophy of the anterior hypophysis which histologically is due to an increase in eosinophilic cells, and the appearance of a much-debated type of cell, the so-called castration cell.

Recently Evans and Simpson[7] have accumulated data which would seem to indicate that "the functional integrity of male and female germ cells is essential for the utilization of the anterior hypophyseal sex hormone."

We may therefore conclude that functional changes in the uterus and ovaries, such as those occurring during pregnancy, and probably in certain pathological complications or sequelae of pregnancy such as hydatid mole and chorioepithelioma should produce stimulus to the anterior lobe of the hypophysis, which in turn might be manifested by hyperactivity of this gland.

That such is the case has been demonstrated by Aschheim and Zondek[1] who have shown that the blood of the pregnant woman contains an abundance of ovarian hormone and of the pituitary hormones, and that they disappear on the second or third day of the puerperium. This would seem to indicate that they are a specific formation, at least in large and demonstrable

amounts, in pregnancy. Nature works with extreme over-production in everything pertaining to propagation, and the organism freely excretes what is not necessary. In the case of the hormones, the massive excretion takes place in the urine. These two hormones are excreted in the urine in a quantitatively different manner.

The amount of the ovarian hormone which is excreted in the urine during pregnancy rises gradually and slowly in the first eight weeks of pregnancy, and then very suddenly until it reaches its climax in the last two months. The amount of the pituitary hormone which is excreted in the urine rises steeply to its climax immediately after conception and remains at its climax from the second week to the middle of pregnancy. From then on it falls very slowly.[1]

If then we have a means for demonstrating the presence of a particular hormone, and that hormone is excreted in the urine in demonstrable amounts during pregnancy only, then the demonstration of that particular hormone in the urine should be equivalent to the demonstration of pregnancy. And indeed it is upon the basis of such a demonstration that the Aschheim-Zondek test for pregnancy, as well as all modifications of the method of demonstration of the hormone of the anterior lobe of the hypophysis rests. Such tests should be designated hormonal tests for pregnancy, since the test rests on the demonstration of the hormone and not upon the demonstration of pregnancy. However, other tests for pregnancy have been devised, the technique of which rested on the demonstration of the ovarian hormone, that of Frank[9] being the most outstanding example. So in order to avoid confusion it is necessary to refer to the test based upon the demonstration of the anterior pituitary secretion as the Aschheim-Zondek test. According to these authors[1] the diagnosis of pregnancy from the urine is an ancient practice of some 3,000 to 4,000 years standing, for it is described in an old Egyptian papyrus "that a woman may determine if she is pregnant by taking some earth and barley in a vessel and adding to it a little of her urine day by day. Should the barley grow, the woman is pregnant, but if the grain does not grow, then she will not bear a child."

The ovarian hormone is negligible in the early diagnosis of pregnancy because its excretion in the urine is not constant in the first eight weeks, and because it is also formed and excreted in functional disturbances of the ovary, and in various other diseases, as climatericum, hyperhormonal amenorrhea, tumors, and so forth.

The hormone of the anterior lobe of the pituitary body, (that is its massive production in the organism and excretion in the urine, up to a thousand times as much as normal) is specific of the earliest stage of pregnancy. Therefore, its demonstration in the urine is a means of early diagnosis.

The work of Aschheim and Zondek amply demonstrates that the diagnosis of pregnancy by means of this test is not only theoretical but practical. They have tested over a thousand specimens of urine with an accuracy of 98.6 per cent. Similar results have been reported from many other laboratories, so that it is perfectly evident that success in its utilization is not confined to the originators of the test. The details of the test have appeared in many papers, and we shall not repeat them here, other than to point out that the animals used were immature female white mice about three weeks of age and weighing between 5 and 8 gms. The injections of urine were made subcutaneously, and in six portions, three the first day and three the second day, and the ovaries were examined for sign of precocious maturity at the end of ninety-six hours. Five immature mice were used for each test.

We began working with this test in the fall of 1929, and soon became aware of a number of difficulties in the technique of the test rather than the end results. The first difficulty naturally was that of obtaining immature white female mice. We may point out that Aschheim and Zondek maintained a colony of 10,000 white mice as the source of their supply. The care of a colony sufficiently large to supply five immature female white mice at the proper stage for each test as needed, entails considerable expense and difficulty. In the second place the test requires about 100 hours for its completion.

Since the test finds its greatest value in differential diagnosis,

and actually makes the Clinical Pathologist a clinical consultant, its value would be greatly enhanced if this time period could be shortened. In hospitalized patients it is frequently not as practical to extend their hospitalization to ninety-six hours as to twenty-four hours, the economic factor being an important item as well as the clinical factor. And third, while the inspection of the mouse ovary in most cases reveals a clean cut positive or negative, in some cases it is difficult to interpret; and the use of five mice for each test, some of which are frequently positive while others are negative, would seem to point to the variation of absorption and utilization of the hormone even when present.

EXPERIMENTS

Encountering these difficulties in using mice in the test, we began experimenting with other animals in the hope of obtaining (1) an animal that could be used at any time, (2) an animal with larger ovaries in order that the end result might be more clean cut macroscopically, (3) an animal in which the end result might be obtained with greater rapidity. We began by working with immature and mature white rats, expecting to continue with the guinea pig and rabbit. We found the results with the immature white rat to be similar to those obtained with the white mice, and to give no improvement in general over the white mice.

It then occurred to us that if Graafian follicles were ripening constantly in immature animals and the hormone of the anterior pituitary was the stimulus needed to bring about ovulation and convert these follicles into corpora lutea, this change might be more readily and rapidly brought about if the hormone were administered to an animal that possessed mature follicles at all times but did not ovulate periodically. There are at least three such animals, namely the rabbit, the ferret, and the cat. In these animals ovulation does not occur until after copulation. In fact it has been demonstrated by Goichi[13] that ovulation occurs within eighteen hours after copulation in the rabbit. Manifestly the rabbit was the most satisfactory laboratory animal of the three.

Since the hormone of the anterior lobe of the pituitary body is

physiologically liberated and distributed by the blood stream, we considered the intravenous administration of the urine logical. Our attention was directed to an article by Friedman[10] in which he recorded the demonstration of the practical application of these principles. He reported a series of thirty-six rabbits which exactly checked with the subsequent clinical course in relation to pregnancy. Friedman's work, while primarily physiological in character, suggested the possibility of applying this test in routine clinical work. It consisted in comparing results obtained by the intraperitoneal and intravenous injection of the urine of pregnant women into non-pregnant does. The summary of Friedmans work is quoted.

1. It has been impossible to produce ovulation in the rabbit by transplantation of as many as 15 fresh rat hypophyses, or by the intraperitoneal injection of 24 fresh rat hypophyses. 2. Intraperitoneal injection of urine from pregnant women produces luteinization of the resulting corpora hemorrhagica in the rabbit ovary. 3. Single intravenous injection of urine from pregnant women provokes ovulation in the rabbit. 4. The samples of urine so far obtained from non-pregnant women have been utterly without effect on the rabbit ovary, either when injected intraperitoneally or when injected intravenously.

It appeared from this work, that the rabbit would supply the practical demands outlined above, if the reliability of the test equaled that of the Aschheim-Zondek test. With this in mind we began using the rabbit for the test, and whenever possible, comparing the results of the tests on rabbits with mice.

The animals should be adult female, non-pregnant rabbits weighing not less than four pounds. The urine from a suspected pregnant woman should be a catheterized morning specimen collected in a sterile container. It should be used as soon as possible, or if it must be retained for an hour or so, should be placed on ice at once, and so preserved until it is used. The amount of urine which we inject is from 5 to 12 cc. and the injection is made intravenously. The reason for the intravenous injection is that the end result is obtained much more rapidly by this method than by intraperitoneal injection. Thus, we may note the end results of the reaction in twenty-four hours after intravenous injection, whereas from four to six days are required

for the intraperitoneal and subcutaneous reactions. The positive reaction consists in the finding of corpora hemorrhagica or of corpora lutea in varying numbers usually in excess of those found in the natural course of ovulation; in other words, an ovulation induced by the substitution therapy.

The economy in the use of animals may be further extended if desired, by performing a laporatomy upon the animal and if negative, suturing the wound and using the same animal for a second test within a week or ten days. Animals, if the test is positive are not satisfactory for re-use, as luteinization and scarring of the ovary distorts it considerably for at least a month, and may result in a false interpretation of the results of a test.

Our experience with this test so far, is limited to a series of fifty cases, which have been checked by subsequent clinical history. Of these fifty cases the hormone diagnosis of pregnancy as revealed by subsequent history or operation has been supported in forty-nine cases. In one case the test was negative and at operation the patient presented the usual signs of pregnancy. The appendix was removed and the abdomen closed. A subsequent test on this patient was strongly positive. We do not know what was the cause of the failure in the first test, but if the urine was not mixed with some other patient's urine, then we must assume either a temporary absence of the hormone from the urine, or the presence of something either eliminated with the urine, or added to the urine after elimination which destroyed the hormone in that specimen. That the difficulty was not in the animal in this particular case is revealed by the fact that when subsequently injected with a known positive urine, there was a positive response. We would like to point out that from the pathological standpoint the investigation of failures with the utmost care is of extreme importance.

The earliest case that we have been able to check definitely is one in which the date of coitus was known. Twenty-one days later, the patient having missed her menstrual period meanwhile, this test for pregnancy was markedly positive. Subsequent observation confirmed the diagnosis.

Another case which proved interesting and at the same time

demonstrated the value of the test, occurred in a sanatorium for tuberculosis. The patient, suffering from advanced tuberculosis when admitted, had missed two menstrual periods. The physician in charge felt it was due to tuberculosis, while the obstetrician believed she was pregnant. The test was positive, and was later proved to be correct.

Gynecologists are not infrequently confronted by patients who have fibroids, become pregnant, and desire a hysterectomy for fibroids, concealing the part of their history which relates to pregnancy, in order that the pregnancy may be interrupted. We have had two cases of this kind, in which while the fibroids were present, the test proved positive for pregnancy, and the gynecologist refused to operate. At the last report both cases were progressing with pregnancy satisfactorily.

We have not had the opportunity of carrying out the test on a hydatidiform mole or a chorioepithelioma. However, Aschheim and Zondek report the value of such tests in these cases. The urine from these patients contains an abundance of the hormone of the anterior pituitary body, and the test is positive. This then provides a means of checking the progress of such a condition as well as a test for retained living placental tissue, and in all cases of miscarriage, abortion, etc., the test should be tried at frequent intervals, and when the test becomes negative, it seems justifiable to assume that there is no more living placental tissue present.

While the number of cases that we are reporting seems rather small in comparison to some of the series reported with mice as experimental animals, we might say that we have worked exclusively on the practical application of a demonstrated physiological fact. All of our cases have been those in which the practical question of clinical differential diagnosis has been involved. We have not run this series with the idea of producing a massive number of cases for statistical purposes. This was not possible with us because we could not secure a sufficiently large number of non-pregnant does to provide such a series. However, we have demonstrated to our own satisfaction, first, that by maintaining constantly a colony of twelve non-pregnant does, we have

sufficient animals to supply the ordinary clinical demands for the test; second, that in this test we seem to have about the same correct diagnostic percentage as in the Aschheim-Zondek test; third, that the intravenous method with rabbits greatly accelerates the time required for the test, and fourth, that in the adult female rabbit we have an animal that may be used at any time.

CONCLUSIONS

In conclusion we desire to emphasize the practical phase that this modification of the Aschheim-Zondek test brings to the Clinical Pathologist. These are (1) accessibility of the proper animal, (2) low cost in the care of the animals, (3) an animal that is available for the test at all times, (4) rapidity of obtaining the diagnosis, and (5) the ease with which the end results are interpreted.

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UNEXPECTED AUTOPSY FINDINGS IN UNEXPECTED DEATHS*

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Death is usually anticipated. Physiological death resulting from senility and the gradual wearing out of organs and tissues is probably an exceptional occurrence. The end usually comes as a culmination of some disease of whose ravages the clinician and the patient are aware. Sometimes it comes with suddenness as the result of an accident, of the imbibition of a poison, or of a sudden turn for the worse in an already existing disease, and sometimes it comes for no apparent reason. It is the unexpected cases of death to which reference is made in this paper.

When one hears of an unexpected death, one's mind usually turns to two, or at most three, of the systems of the body. One thinks first perhaps of the circulatory system, and secondarily of the nervous and respiratory systems, and as a matter of fact, the majority of cases of unexpected death may be referred to one of these. But in the course of autopsy experience one learns that there is a wide range, in lesions of these systems, and that the primary cause of the demise may occasionally originate in other systems and tissues, although the actual termination of life may be due to the effect upon one of the three systems previously mentioned. The cases reported have, with a few exceptions been selected from a series of about 1200 autopsies performed at the Hamilton General Hospital during the past ten years. Many of the more unusual observations have been revealed in autopsies done on cases, which have been the subjects of coroner's investigations, and where little or no previous clinical history was available. No doubt some of the so-called *unexpected* findings

would have been *expected* had the cases been studied clinically before hand, but that is a condition which does not, as a rule, obtain in cases of unforeseen death.

The most common lesions of the heart which result in unexpected death are myocardial degeneration, coronary thrombosis or embolism, and infarction of the heart muscle. The following cases, in my experience at least, are unusual.

LESIONS OF THE CIRCULATORY SYSTEM

Extreme fatty degeneration of heart muscle

A young girl has contracted syphilis at the age of seventeen years. She had been treated with arsenicals in the out-patient department of the hospital, and after the second course of treatment had developed jaundice and for this reason had been admitted to the ward. The jaundice disappeared and the condition apparently improved. On the day before she was to have been discharged, she got out of bed to go to the toilet, as she had been doing for some days, and on the way collapsed and died in about fifteen minutes. At autopsy, the liver was found to be enlarged and fatty, and the heart weighed 350 grams and in appearance and on section was like putty. Sections showed an advanced degree of fatty degeneration of the myocardium. No other gross pathological condition was found sufficient to account for death. Apparently some undue exertion had caused interference with the conducting bundles and had caused the sudden demise. The comparative youth of the patient is the interesting factor.

Traumatic hematopericardium

A farm worker, aged twenty-four years took pleasure in teasing a lad aged fourteen years who was a summer visitor on the farm. In the course of an altercation, the boy picked up a pitchfork and hurled it from a distance of a few feet at the hired man. The man gave a cry of pain but did not seem to be seriously hurt. In a few minutes, however, he complained of feeling faint and a physician was called, who, on arrival a short while later found

him breathing his last. Physical examination showed a very small puncture wound between the second and third ribs on the right side with no bleeding. A coroner was notified who ordered an autopsy. Upon examination, it was found that one prong of the fork, which was small, had penetrated through the lung, through the upper part of the pericardium and had torn a hole in the aorta about 1 cm. in length, from which blood had been pumped until the pericardium was distended to a degree which caused cessation of the heart's action.

The same finding was observed in another case where the wound was caused by the blade of a jack-knife in a stabbing affray between a colored man and his paramour, the former being the victim.

Aortic stenosis

A man aged forty-five years was found dead in bed at his rooming house. No history of recent previous illness could be elicited. At autopsy, an hypertrophied heart weighing 450 grams and with a marked stenosis of the aortic valve was found. No other lesions not related to this were discovered. It is probable that in cases like this one the fibrosis of the myocardium which accompanies the hypertrophy may finally interfere with one or the other of the conducting bundles of the heart.

Congenital absence of ventricular septum

The usual congenital abnormalities found are patent foramen ovale, patent ductus arteriosus, and patent ductus venosus. In this case of a new-born infant which was poorly nourished and which lived only three days, there was a congenital absence of the ventricular septum which gave the child a three-chambered heart.

Coronary thrombosis at fourteen years

A young Polish girl, aged fourteen years, skipped 300 times at school in the morning and had a pain in the precordial region, which passed away. At noon she began her lunch, had a return of the pain, vomited, and died in five minutes. An autopsy was

ordered by the coroner. The aorta was found to show a marked degree of atheroma, some of the patches showing ulceration. The mouth of the left coronary artery was almost occluded by vegetations. The first 2 cm. of the coronary artery were blocked by a thrombus and the surrounding tissues in this area showed inflammatory change. On going into the history, it was found that the girl had had attacks of scarlet fever, measles, chicken pox, diphtheria and whooping cough, since the age of six. The interesting point about this case is the age.

LESIONS OF THE RESPIRATORY SYSTEM

Lobar pneumonia

A street cleaner, aged about sixty years, had been in the city employ for about five years. One day he collapsed on the street and was brought to the hospital in the police ambulance. He died about one hour after admission. At autopsy lobar pneumonia involving the whole of the right lung in the gray hepatization stage was found. The heart showed considerable myocardial degeneration. The man had been apparently carrying on his work while suffering from lobar pneumonia for several days.

Influenza pneumonia

A man aged about forty-five years, had been actively carrying on his work as a manager. One morning he felt chilly but carried on his work until lunch time. After lunch he became quite ill, finally going into coma and dying a few hours later. At autopsy both lungs were found to be involved by a hemorrhagic type of bronchopneumonia from which Streptococcus (hemolytic) was cultured. There was also some edema of the lungs. The man had apparently suffered from a fulminating attack of bronchopneumonia of the influenzal type.

LESIONS OF THE NERVOUS SYSTEM

Cerebellar tumor

A patient in the Hamilton General Hospital had been brought in for diagnosis with a tentative diagnosis of brain tumor. A

diagnostic lumbar puncture was performed and 12 to 15 cc. of clear spinal fluid was removed. The patient went into convulsions and died within an hour. At autopsy a glioma of the cerebellum was found which had been pressed down into the foramen magnum so as to leave the imprint of the foramen magnum on it. The disturbance of pressure brought about by lumbar puncture had apparently caused sudden death.

Hemorrhage into cerebral cyst

A workman, aged twenty-four years, on his way to work one morning bumped his head slightly on the top of the car when getting into it. In a few minutes he lapsed into unconsciousness and was taken to a doctor's office from where he was sent to the hospital dying shortly after admission. At autopsy there was found a large blood-filled cyst of the anterior part of the right cerebral hemisphere. This cyst was roughly 8 cm. by 6 cm. There was no evidence of neoplastic tissue in the walls of the cyst. Apparently the slight blow which left no mark on the skin was sufficient to cause a rupture of a vessel bordering on the cyst. The hemorrhage had burst into the right lateral ventricle.

Death in epilepsy

A gentleman was found in his apartment one morning dead with his face buried in his pillow. Apparently he had been well the night before. At autopsy there was marked congestion of the face and petechial hemorrhages scattered over the neck, arms, and chest. Internally there were the usual evidences of suffocation. It was hard to understand how an apparently healthy man of forty could suffocate in this manner. He had explained previous periods of illness by stating that he had had a kidney removed during the war and suffered from renal insufficiency. There was no evidence at autopsy of a kidney wound or operation; both kidneys were of normal size and appearance except for congestion. The history, secured later, showed that he had been suffering from epilepsy and used this story to explain periods of indisposition. He died from suffocation in an epileptic attack.

LESIONS OF THE ALIMENTARY SYSTEM

Appendiceal hemorrhage

A young woman, aged twenty-five years, was brought into the hospital, having been ill for three days and having passed blood from the bowel on the day before. She was very anemic. A diagnosis of typhoid fever was made but Widal reactions were negative. She died on the day following admission. At autopsy the appendix was thickened and ulcerated and imbedded in a mass of adhesions. Dissection showed that it contained a small black-headed pin about 2 cm. in length. Ulceration had taken place but without external rupture of the appendix. The lumen of the appendix was patent and had served as a drainage tube for blood coming from an eroded artery. The patient died of hemorrhage.

Infected Meckel's diverticulum

A young man, aged twenty-three years, was admitted to the hospital with a diagnosis of general peritonitis. A drain was inserted but he died on the third day after admission. At autopsy a small piece of impacted fecal material was found in a badly ulcerated Meckel's diverticulum and this appeared to be the starting point of the infection.

Traumatic rupture of spleen

A young soldier in Macedonia during the late war had had repeated attacks of malaria. He was thrown from his horse one day and went into a state of collapse from which he did not recover. He died the next day. At autopsy the spleen weighed 2000 grams and had ruptured from the fall.

LESIONS OF THE GENITO-URINARY SYSTEM

Unexpected death occasionally occurs from acute uremia. A boy, aged sixteen years, in coma was admitted from a boy's home. At the time no history of nephritis could be obtained. He died within forty-eight hours of acute uremia. At autopsy, contracted kidneys were discovered. Further inquiry elicited

the fact that the boy at the age of eight years had had a very mild attack of scarlet fever.

STATUS LYMPHATICUS

Every pathologist must carry in his mind this condition in cases of unexpected death in young people. The following case illustrates this condition:

A boy, aged twelve years, was in a boat with several older boys. An altercation arose and one of the boys struck at him with an oar. He fell overboard and never rose to the surface. The body was recovered some hours later. Autopsy showed no evidence of injury and absolutely none of the evidences of drowning. It did reveal, however, the classical anatomical evidence of status thymico-lymphaticus. The thymus weighed 45 grams. There was great hyperplasia of the lymphoid elements, especially of the mesenteric glands, and the lymphatics of the colon, with a relative hypoplasia of the heart and aorta. The boy had apparently succumbed to the shock occasioned by sudden fear.

VIOLENT DEATHS

Rupture of duodenum

A workman in a sand pit was crushed between the wall of the pit and the hub of a wagon. He was admitted to the hospital, and two days later showed symptoms of intestinal obstruction. No operation was performed and he died the next day. At autopsy there was no peritonitis but dissection of the duodenum showed it to have been torn across by being pinched against the vertebral column. The surrounding retroperitoneal tissues were inflammatory and to some extent infiltrated with duodenal contents. There was a small hematoma at the root of the mesentery.

Bullet in aorta

A workman was shot through the chest in a brawl. Post-mortem examination showed the bullet to have traversed the right side of the chest, struck the body of the fifth thoracic vertebra, and made a hole at this point in the aorta. It was found in the aorta at a point about 3 cm. above the bifurcation.

Acute pulmonary edema

In several accidents, death has been caused by the onset of edema of the lungs where the injuries seemed trivial. It is probably associated with nervous system shock.

No doubt many pathologists have seen cases similar to these reported but one should always remember that sudden death can be brought about by a wide variety of causes arising in almost any one of the organs or systems of the body. Attention directed to unusual cases of this kind is always of benefit.

ADDITIONAL OBSERVATIONS ON ISOLATING TUBERCLE BACILLI

The Oxalic Acid Reagent for Primary Culture*

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In a study reported last year (Corper and Uyei[1]) as part of a plan to improve and elaborate upon practical methods for the certified diagnosis of tuberculosis it was found that a 5 per cent oxalic acid reagent might possibly be substituted with advantage for the 6 per cent sulphuric acid reagent originally recommended in the new sulphuric acid-crystal violet potato medium method for isolating tubercle bacilli from tuberculous materials (Corper and Uyei[2,3,4]).

In the report it was shown that the 5 per cent oxalic acid reagent was superior to the six per cent sulphuric acid reagent for isolating tubercle bacilli from tuberculous sputums in that a greater percentage of the total tubes planted yielded positive cultures of tubercle bacilli, this being accounted for by the fact that the oxalic acid reagent was less toxic to tubercle bacilli as determined by bacteriostatic tests and also by the fact that the oxalic acid reagent possessed a greater germicidal action for the contaminating organisms found in sputum. Although the foregoing results obtained with tuberculous sputums and the results of the bacteriostatic experiments were in favor of the oxalic acid reagent over the sulphuric acid reagent, we still hesitated to recommend the oxalic acid reagent at that time to replace the sulphuric acid reagent in the new method of cultivating tubercle bacilli until

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† Dr. H. J. Corper was awarded the Ward Burdick Medal for his work on the cultivation of tubercle bacilli.

more data with a greater variety of materials and specimens had been obtained.

It is the purpose of this contribution to report such studies with urines and contaminated tuberculous animal tissues which corroborates the previous findings with sputums in indicating that the 5 per cent oxalic acid reagent can be used to replace the 6 per cent sulphuric acid reagent in the sulphuric acid-crystal violet potato cylinder method for cultivating tubercle bacilli from tuberculous materials if desired.

TABLE 1

A Comparison of the 5 Per Cent Oxalic Acid Reagent with the 6 Per Cent Sulphuric Acid Reagent for Isolating Tubercle Bacilli from Urine

REAGENT USED FOR DESTROYING CONTAMINATORS	NUMBER OF TUBES OF POTATO MEDIUM PLANTED	NUMBER OF TUBES THAT BECAME CONTAMINATED	NUMBER OF TUBES IN WHICH TUBERCLE BACILLI WERE ISOLATED	PERCENTAGE CONTAMINATIONS	PERCENTAGE ISOLATIONS
5 per cent oxalic acid........	144*	52	37	36	26
6 per cent sulphuric acid.....	144	59	36	41	25

* Twenty-four specimens of urine were tested and each urine planted on six tubes of glycerol water-crystal violet-potato cylinder medium.

I. A COMPARISON OF OXALIC ACID WITH SULPHURIC ACID FOR THE ISOLATION OF TUBERCLE BACILLI FROM TUBERCULOUS URINES

In this experiment, twenty-four specimens of urine were used. These urines were obtained from patients suspected of having renal tuberculosis or known to have renal tuberculosis. The urines were tested using the technic previously described by us for isolating tubercle bacilli from tuberculous materials; as reagents for destroying contaminators either the 6 per cent sulphuric acid reagent or the 5 per cent oxalic acid reagent were used in comparative tests. After treating the sediment of urine specimens with an equal volume (one cubic centimeter) of the acid reagent and diluting with sterile saline solution after thirty minutes incubation with occasional shaking, the washed sediment was planted on six tubes of glycerol crystal violet potato medium for

each specimen of urine and for each of the acid reagents tested. Thus one hundred and forty-four tubes of medium were planted from the twenty-four urine specimens for each reagent tested. The planted potato culture tubes were incubated at 37°C. and at weekly intervals were examined and the amount of growth found was recorded.   All positive macroscopic findings were checked by examining smears stained by the Ziehl-Neelsen method for the presence of acid-fast bacilli.   The results of these findings are recorded in table 1.

The findings recorded in table 1 indicate that there is a slight superiority of the 5 per cent oxalic acid reagent over the 6 per cent sulphuric acid reagent in that the use of the former with urines results in a smaller percentage of contaminations and a slightly greater percentage of isolations of tubercle bacilli.   This agrees well with the results recorded previously for tuberculous sputums in which the 5 per cent oxalic acid reagent yielded 88 per cent isolations of tubercle bacilli and 15 per cent contaminations, as compared to 78 per cent isolations of tubercle bacilli and 28 per cent contaminations with the 6 per cent sulphuric acid reagent.

II. THE ISOLATION OF TUBERCLE BACILLI FROM INFECTED TISSUES WITH THE OXALIC ACID REAGENT OR THE SULPHURIC ACID REAGENT

In this experiment tissues were obtained from four dogs and four rabbits previously given intravenous injections of different amounts of fine suspensions of virulent human tubercle bacilli (Gluckson).   After a lapse of four to fourteen days after the injection of the tubercle bacilli, the animals were killed by electrocution and specimens of five different tissues, namely, lung, liver, spleen, kidney, and bone marrow, from each animal were used for the isolation of the tubercle bacilli from the tissues.   A specimen of the tissue usually a piece about the size of a pea was finely ground up in a porcelain mortar using about three to five cubic centimeters of 0.9 per cent saline solution to obtain a good mixture.   About 1 cc. of the tissue suspension in the saline solution, as free as possible from large lumps, was submitted to the

routine isolation technique for tubercle bacilli using either an equal volume (1 cc.) of the 5 per cent oxalic acid reagent or of the 6 per cent sulphuric acid reagent and after incubation and dilution

TABLE 2

A Comparison of the Oxalic Acid Reagent with the Sulphuric Acid Reagent for the Isolation of Tubercle Bacilli from Infected Tissues of the Dog and Rabbit

ANIMAL USED FOR INTRAVENOUS INFECTION	WEIGHT OF ANIMAL	AMOUNT OF BACILLI INJECTED PER POUND BODY WEIGHT	REAGENT	TISSUES FROM WHICH TUBERCLE BACILLI WERE ISOLATED AND NUMBER OF CULTURE TUBES (OF A TOTAL OF FIVE USED) WHICH PROVED POSITIVE FOR TUBERCLE BACILLI				
				Lung	Liver	Spleen	Kidney	Bone marrow
	pounds	*mgm.*						
Dog........	30	1.0	Oxalic	4	5	5	5	5
			Sulphuric	5	4	5	5	5
	27	0.01	Oxalic	4	5	4	4	0
			Sulphuric	3	5	5	5	0
	18	0.000,1	Oxalic	3	2	—*	0	1
			Sulphuric	2	5	—*	0	1
	28	0.000,001	Oxalic	2	4	2	1	4
			Sulphuric	1	5	2	0	4
Rabbit.....	9	1.0	Oxalic	3	5	5	5	5
			Sulphuric	3	5	5	5	5
	7.5	0.01	Oxalic	4	5	5	5	5
			Sulphuric	1	5	4	5	4
	8	0.000,1	Oxalic	4	3	4	0	5
			Sulphuric	4	2	4	0	5
	9.3	0.000,001	Oxalic	1	3	4	3	4
			Sulphuric	2	2	3	4	4

* The spleen from this dog was not studied.

with saline solution, the centrifugate planted upon the glycerol water-crystal violet potato medium and incubated. The quantity of tubercle bacilli injected intravenously into the dogs or

rabbits varied from 1.0 milligram to 0.000,001 mgm. per pound body weight of the animal that was injected.

The findings in this experiment are recorded in table 2 as the number of potato culture tubes found positive from each specimen of tissue taken from the different organs, following the use of either the oxalic acid or sulphuric acid reagent; a maximum of five tubes having been planted for each specimen of tissue taken and submitted to examination for tubercle bacilli.

The results of the study with infected animal tissues recorded in detail in table 2 become more evident when the findings are summarized as given in table 3 and when the number and percentage of contaminations are also noted.

TABLE 3

SUMMARY OF COMPARISON OF THE OXALIC ACID REAGENT WITH THE SULPHURIC ACID REAGENT FOR THE ISOLATION OF TUBERCLE BACILLI FROM TUBERCULOUS ANIMAL TISSUES

REAGENT USED IN EQUAL VOLUME	TOTAL NUMBER OF POTATO CULTURE TUBES USED FOR 39 TISSUE SPECIMENS TESTED	NUMBER OF TUBES POSITIVE FOR TUBERCLE BACILLI	NUMBER OF TUBES CONTAMINATED	PER CENT ISOLATION OF TUBERCLE BACILLI	PER CENT CONTAMINATIONS
5 per cent oxalic acid........	195	138	26	71	13.8
6 per cent sulphuric acid. ...	195	134	39	69	20.0

It is evident from the results recorded in tables 2 and 3 that the 5 per cent oxalic acid reagent possesses a slight but definite advantage over the 6 per cent sulphuric acid reagent for isolating tubercle bacilli from infected tissues. The results are not so evident when the number of positive findings for tubercle bacilli are considered from the standpoint of the tissues tested as when the number of positive culture tubes are noted. It is especially significant that the number of contaminated tubes was higher with the sulphuric acid reagent than with the oxalic acid reagent. These findings with tissues corroborate those previously recorded with sputums and bear out the findings with urines in indicating that the 5 per cent oxalic acid reagent possesses a decided, though slight, advantage over the 6 per cent sulphuric acid reagent for

destroying contaminators and increasing the number of positive cultures obtained from the use of the acid glycerol water-crystal violet potato cylinder method for detecting tubercle bacilli in suspected tuberculous materials.

III. THE ISOLATION OF BOVINE TUBERCLE BACILLI

In our earlier experiments performed to perfect the method of isolating tubercle bacilli from tuberculous materials when present in small numbers the magnitude of the problem prevented an exhaustive study with bovine tubercle bacilli and most of the tests with this organism were restricted to the use of a number of laboratory strains which had been under artificial cultivation for some years. Although it was noted at that time that the potato medium supported the growth of small numbers of bovine tubercle bacilli slightly less efficiently than it did human tubercle bacilli, the differences were not especially striking and particularly so since the potato medium was found to be the best nutrient medium, of all the mediums tested, for supporting the growth of small numbers of bovine tubercle bacilli. Shortly after the glycerol water-crystal violet-potato method was described by us Dr. William H. Feldman[c] found in trying to use this method for isolating bovine tubercle bacilli that it did not give as gratifying results as when isolating human tubercle bacilli and asked us to corroborate his findings and if possible find out the reason for this difference in behavior of the bovine and human bacilli. Accordingly strictly bovine specimens were immediately obtained from the government inspectors[*] examining cattle at the Denver Packing Houses.

In this experiment was included an examination of sixteen specimens taken from various organs in definitely tuberculous cattle as they came from the Denver slaughter houses on the day of inspection immediately after killing the cattle. The technique followed for isolation was identical to that previously reported elsewhere for the oxalic or sulphuric acid glycerol water-crystal

violet-potato cylinder medium method. Two reagents, namely 6 per cent sulphuric acid or 5 per cent oxalic acid were used for preliminary sterilizing to remove the contaminators. Six culture tubes containing glycerol water-crystal violet-potato cylinder medium were used for planting from each specimen of tissue, so that in all 96 tubes were used for each acid reagent. In addition to testing by the culture method, two guinea pigs were given the ground tissue material by subcutaneous injection in order to compare the efficiency of the culture method with the guinea pig method for the detection of bovine tubercle bacilli in bovine material.

Out of sixteen tissue specimens used, which included lung, liver, bronchial, mediastinal and mesenteric glands, seven specimens proved positive by the sulphuric acid reagent method and nine specimens proved positive by the oxalic acid reagent method, while all the specimens were positive by guinea pig inoculation. On the basis of the culture tubes planted, the sulphuric acid reagent method revealed twenty tubes out of ninety-six or 21 per cent positive isolations, while the oxalic acid reagent revealed twenty-four tubes out of ninety-six or 25 per cent positive isolations.

It is natural that the question of the cause of these inferior results with the culture method should merit elucidation in order to attempt to remedy the deficiency if possible and better adapt the culture method to include the detection of bovine bacilli. Naturally the solution resolves itself to determining whether the poor results are due to the toxicity of the reagents used to destroy the contaminators or whether the deficiency of the medium is accountable, the latter being suggested as a result of the earlier studies with laboratory strains of bovine bacilli.

In order to determine whether the toxicity of the acid reagents was accountable, the effect of 6 per cent sulphuric acid, 5 per cent oxalic acid and 3 per cent acetic acid on freshly isolated bovine tubercle bacilli was studied. For this purpose one cubic centimeter of a finely divided supension of recently isolated bovine tubercle bacilli* containing one milligram, 0.01 or 0.0001 mgm. were mixed

* Some of the strains used in this and subsequent experiments were obtained from Dr. H. C. Sweany of the City of Chicago Municipal Tuberculosis Sanitarium, and from Dr. Wm. H. Feldman of the Mayo Foundation.

with one cubic centimeter of the reagents above being tested. After incubation of thirty minutes at 37°C. the mixture was diluted to ten cubic centimeters with sterile saline solution, centrifugated, decanted and the residual material was planted on tubes of the glycerol water-crystal violet-potato cylinder medium. The growth obtained at incubator temperature (37°C.) was compared with the growth obtained in control culture tubes in which the tubercle bacilli were planted after treatment with 0.9 per cent saline solution alone without the use of the acid reagents.

The findings in this experiment revealed no difference in the growth of bacilli on the control tubes as compared to the experimental tubes in which the bacilli had received the preliminary treatment with the oxalic or sulphuric acid reagents, indicating that these reagents are practically innocuous to the recently isolated bovine tubercle bacilli in the concentrations and manner tested which is that outlined in the oxalic or sulphuric acid glycerol water-crystal violet-potato cylinder method. This lack of harmful effect of the acids upon the bacilli in concentrations of an equal volume of 5 per cent oxalic acid or 6 per cent sulphuric acid was also confirmed by treating the tuberculous bovine specimens with either of these reagents and injecting the saline washed sediment into guinea pigs and comparing the findings with those obtained by inoculating guinea pigs in similar manner with the untreated specimen of tuberculous tissues. The tuberculosis developed in the controls and in the test guinea pigs to a like extent. These findings would therefore appear not to incriminate the oxalic acid reagent or the sulphuric acid reagent in accounting for the unsatisfactory results obtained in isolating bovine tubercle bacilli from fresh bovine tuberculous materials. There remains, therefore, the problem of determining whether the deficiency of the medium as a nutrient for small numbers of bovine tubercle bacilli can account for the unsatisfactory results obtained. This experiment was rather extensively pursued and can be divided into two parts; one (a) to note whether glycerol was detrimental and what concentrations if any could be used for growing small numbers of bovine tubercle bacilli and the other (b) to determine the comparative nutrient value of various mediums for growing bovine tubercle bacilli.

a. Determination of the optimum concentration of glycerol in the medium essential to growing the bovine tubercle bacillus

The optimum concentration of glycerol for growing human strains of tubercle bacilli when the latter are present in small numbers was previously found to be about 2 to 3 per cent concentration in the potato nutrient medium. The concentration of glycerol used in the crystal violet potato cylinder medium was so adjusted that the final concentration (using 6 per cent glycerol water) after introducing the planting fluid, etc. was 2 to 3 per cent.

The optimum concentration of glycerol for the growth of laboratory strains (several years of artificial cultivation) of bovine tubercle bacilli was previously (Corper and Uyei[4]) found to be about 2 per cent in potato broth agar medium. Since there is a possibility of change in glycerophilic property of tubercle bacilli, especially during long artificial cultivation in the laboratory, the optimal glycerol concentration in the medium for the growth of small numbers of recently isolated strains of bovine tubercle bacilli was also determined.

In this experiment one cubic centimeter of pure glycerol varying in concentration from 0.0 to 10 per cent in distilled water was used in the crystal violet potato cylinder medium, and on this medium was planted varying amounts of a number of recently isolated bovine strains of tubercle bacilli in fine suspensions.

It was surprising to note that all the recently isolated strains of bovine tubercle bacilli tested grew equally well in the potato mediums regardless of the amounts of glycerol added from 0.0 to 10 per cent showing that a wide range of glycerol can be used and that this factor is not pertinent for the isolation of small numbers of bovine tubercle bacilli on the potato cylinder medium. In order to further confirm this point experiments are now in progress to determine the effect of glycerol in the potato medium on the isolation of bovine tubercle bacilli from infected tissues.

b. A comparative study of various mediums as nutrients for the growth of bovine tubercle bacilli

A study of various mediums as nutrients for small numbers of recently isolated bovine tubercle bacilli has not advanced far

enough to make a definite report and therefore only the general plan of this phase of the study will be submitted, reserving the report of the results obtained for a subsequent article.

Among the mediums in contemplation for use and already under test for determining their nutrient value for growing small numbers of recently isolated strains of bovine tubercle bacilli and for isolating small numbers of bacilli from bovine tuberculous materials are included glycerol broth-gentian violet egg medium (Petroff), glycerol broth egg medium (Dorset), Evanoff-Sweany's[5] cream medium, glycerol water-crystal violet-potato medium, plain sweet potato cylinder medium, glycerol water sweet potato cylinder medium, Jerusalem artichoke cylinder medium (with and without glycerol), potato milk medium, potato cream medium and others.

It is hoped thus by studying the nutritive value of various new substances, as well as suggestive combinations, to increase the culture efficiency especially for determining the presence of small numbers of tubercle bacilli in bovine tuberculous materials and finally if possible to devise a simple single medium to serve for both human and bovine tuberculous materials.

SUMMARY

In verification of previous findings reported for sputums it was found that a 5 per cent oxalic acid reagent, for destroying contaminators in the culture method for the diagnosis of tuberculosis, also proved slightly superior to a 6 per cent sulphuric acid reagent with specimens of urines and infected animal tissues. The superiority of the oxalic acid reagent expressed itself in a slightly higher percentage of positive culture tube findings in addition to a lower percentage of tubes contaminated and a lesser detrimental effect in comparative toxicity tests with suspensions of tubercle bacilli.

The use of the oxalic or sulphuric acid glycerol water crystal-violet potato cylinder method for detecting the presence of bovine tubercle bacilli in tissues was less satisfactory than for detecting human bacilli. The explanation for this resides in the inadequacy of the potato medium and not in the oxalic or sulphuric acid reagents used for destroying contaminators nor in the glycerol used

as part of the glycerol water crystal-violet potato cylinder medium. The potato medium has thus far proved to be the best medium, of the mediums tested, for growing small numbers of bovine tubercle bacilli and investigations are now in progress to find a more suitable nutrient for the bovine tubercle bacilli present in bovine tuberculous tissues.

Appreciation is due L. D. Miller and Margaret Uyei for assisting with the technical phases of this study.

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THE DIRECT CALCULATION OF THE VOLUME AND HEMOGLOBIN CONTENT OF THE ERYTHROCYTE*

A Comparison with Color Index, Volume Index and Saturation Index Determinations

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Knowledge of physical variations in the erythrocyte which take place in the various anemias has been much hampered by the inaccuracy and inadequacy of hematologic technic. These defects until recently have passed unchallenged and even today the gross errors of the commonly used methods of estimation of hemoglobin and the wide variation in the gram-equivalent accepted as 100 per cent or "normal" for the various hemoglobinometers is by no means generally appreciated. Advances in our knowledge have been further impeded by a lack of sufficiently well founded standards of the normal. It is only in the past few years that an attempt has been made to supply accurate standards for the normal red cell count, hemoglobin and volume of packed red cells [9,10,13,15,17,18].

The color index affords a good example of the confusion and inaccuracy which is quite common. This index is meant to express in a given case the relative amounts of hemoglobin and erythrocytes in proportion to the normal, or, what is the same, the average hemoglobin content of the erythrocytes of the sample in proportion to the hemoglobin content of the erythrocytes of normal blood. For this index to be of any value it is essential that the technic of the hemoglobin and erythrocyte determinations which enter into its calculation be accurate and, furthermore,

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it is necessary that correct standards for normal be employed. Generally speaking neither of these requisites is met in the every day color index determination. The hemoglobinometer employed is frequently inaccurate and furthermore, 100 per cent or normal, as given by the various instruments, represents a wide range of values which vary from 13.8 grams of hemoglobin per 100 cc. of blood on the Dare instrument to 17.2 grams on the Sahli. The marked discrepancy in the value accepted as 100 per cent reflects the inaccurate foundation on which our conception of the normal has been based.

Further error arises from the fact that recently made accurate erythrocyte counts in healthy individuals[13,17,18] indicate that the value which has been assumed to be normal or 100 per cent in the calculation of color index, namely five million erythrocytes, is not correct, the average erythrocyte count in normal men being distinctly greater than this figure. Again, the fact that the "normal" appreciably differs in the two sexes, is another obvious source of error.

The term "color index" is in fact an unfortunate choice and one of the most important arguments which can be brought against this index is the erroneous conception to which the term has given rise. Because in pernicious anemia the color index is greater than 1 whereas in the "secondary" anemias this index is low, there is a general impression that in the former disease the erythrocyte is supersaturated with hemoglobin, whereas in the "secondary" anemias the reverse is found. Color index, however, does not measure the hemoglobin saturation of cells, but rather their hemoglobin content. Direct calculations of the volume and hemoglobin content of the erythrocyte indicate that in anemia one of the essential changes is an alteration in the average size of the erythrocytes. Increases or decreases in the amount of hemoglobin contained in the cells are, with the exception of certain types of anemia, proportional to alterations in the size of the cells[13,22,14,12]. It should perhaps be pointed out that direct calculations of the volume and hemoglobin content of erythrocytes, as well as the various indices, refer only to mean values and give no information concerning the individual cells. Bearing

this in mind it can be said that supersaturation does not occur in pernicious anemia[5,20]. In fact, it is probably true that the average erythrocyte in disease never contains more hemoglobin per unit volume than is found in the normal corpuscle. The erroneous conception regarding the hemoglobin content of the erythrocytes in the anemias may be directly attributed to the use of such a vague and inaccurate term as "color index."

The volume index of Capps,[3] and the saturation index of Haden[6] suffer from imperfections similar to those of the color index. These defects cause me to favor the direct calculation of

TABLE 1

RELATION BETWEEN CORPUSCULAR CONSTANTS AND THE INDICES

Color index $\} =$	$\left\{\dfrac{\text{Hemoglobin per cent}}{\text{Erythrocytes per cent}}\right.$	Mean corpuscular hemoglobin (in micromicrograms) $\} =$	$\left\{\dfrac{\text{Hemoglobin (gm. per 1000 cc.)}}{\text{Erythrocytes (millions per cu. mm.)}}\right.$
Volume index $\} =$	$\left\{\dfrac{\begin{array}{c}\text{Volume packed erythrocytes per cent}\end{array}}{\text{Erythrocytes per cent}}\right.$	Mean corpuscular volume (in cubic microns) $\} =$	$\left\{\dfrac{\begin{array}{c}\text{Volume packed erythrocytes (cc. per 1000 cc.)}\end{array}}{\text{Erythrocytes (millions per cu. mm.)}}\right.$
Saturation index $\} =$	$\left\{\dfrac{\text{Hemoglobin per cent}}{\begin{array}{c}\text{Volume packed erythrocytes per cent}\end{array}}\right.$	Mean corpuscular hemoglobin concentration (in per cent) $\} =$	$\left\{\dfrac{\text{Hemoglobin (gm. per 100 cc.)} \times 100}{\begin{array}{c}\text{Volume packed erythrocytes (cc. per 100 cc.)}\end{array}}\right.$

the volume and hemoglobin content of the red corpuscle. As pointed out by Haden[5] from information concerning the number of erythrocytes and the amount of hemoglobin in a given sample of blood, the actual amount of hemoglobin in the average erythrocyte may be calculated by simply dividing the amount of hemoglobin per unit volume of blood by the number of erythrocytes in the same volume. Similarly the average volume of the red cells of any sample of blood may be calculated by dividing the volume of packed red cells per unit volume of blood by the number of red cells per unit of volume[5,14]. The concentration of hemoglobin in the average cell of the sample of blood is calculated in an equally simple manner by dividing the amount of hemoglobin by

the volume of packed erythrocytes per unit of volume. These direct calculations of the volume and hemoglobin content of the erythrocyte are simple and clear as to their import. They afford a much clearer and more accurate conception of the physical state of the erythrocyte in health and the alterations associated with disease than can be gained by the consideration of the indices. The direct calculations express the same relationship between hemoglobin, volume of packed erythrocytes, and number of erythrocytes as do the indices (Table 1), except that the latter express this relationship in proportion to a supposed normal. As has already been pointed out, the values taken as normal are incorrect and besides, since a single value is taken as the equivalent of 100 per cent, the normal inter-individual variation in hemoglobin, number of erythrocytes and volume of packed erythrocytes is disregarded. I believe, therefore, that this expression in relation to "normal" may well be sacrificed for the sake of greater clearness and accuracy.

The desirability of the direct calculation of the hemoglobin content of the erythrocyte is further supported by the growing tendency to report hemoglobin directly in grams per 100 cc. of blood. This manner of reporting hemoglobin has been proposed in order to avoid the confusion arising from the use of a large number of different values as the equivalent of 100 per cent and is meeting with more and more favor both in the laboratory and at the bedside. In the calculation of color index from data concerning amount of hemoglobin, reported in grams, it is first necessary to convert the hemoglobin to terms of percentage, a step which is unnecessary when direct calculations are made.

In the present paper, simple methods for the calculation of the volume and hemoglobin content of the erythrocyte will be given and normal values for men and women based on accurate blood determinations which I[11,15,17] have carried out, as well as on blood determinations reported by other investigators will be presented. Details of the methods in the determination of number of erythrocytes, amount of hemoglobin and volume of packed erythrocytes, as well as an analysis of their accuracy, are fully elaborated in earlier papers and need not be repeated here.

In the choice of names for the corpuscular constants discussed in this paper the attempt has been made to devise terms which are simple and yet descriptive. It is particularly difficult to find a suitable term to denote the volume of the individual red corpuscle since confusion so readily occurs with the term sometimes used to refer to the volume of packed red cells, namely, "cell-volume." The term "individual cell volume" is used by Haden[7]. This is still somewhat confusing. The terms which I propose, namely mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, are presented because I believe they best fulfil the requisites of simplicity, clarity and uniformity.

MEAN CORPUSCULAR VOLUME

The volume of the average erythrocyte, or "mean corpuscular volume," may be calculated for any given sample of blood by dividing the volume of packed erythrocytes, expressed as cubic centimeters of red cells per 1000 cc. of blood, by the number of erythrocytes expressed as millions per cubic millimeter. The result expresses mean corpuscular volume in cubic microns.

The volume of packed red cells may be determined by centrifugalizing a measured quantity of blood in a suitable hematocrit[16]. This instrument, once so popular, has not received the general usage in the clinical laboratory which it merits. Its obvious disadvantage is the fact that relatively expensive mechanical means are required to secure adequate centrifugalization. This fact, coupled with a general lack of appreciation of the valuable and accurate information which the instrument affords, probably explains why the popularity of the hematocrit was so short lived. This is indeed unfortunate because with reasonable care in technic the determination of relative cell volume by the hematocrit method is very accurate. In the blood determinations which I[13,15,17] reported, in spite of the special attention paid to accuracy, analysis of the methods employed showed that the probable error of the erythrocyte counts was 2 per cent and that of the hemoglobin determinations possibly even as high as 10 or 12 per cent, whereas the probable error of the hematocrit determinations was

0.5 per cent. Since the information derived from hematocrit determinations is to a large extent similar to that afforded by the determination of hemoglobin, in the face of the prevailing inaccurate methods of hemoglobin determination, the advantages of the hematocrit greatly outweigh any possible disadvantages.

It has been stated that it is very doubtful whether any accurate measure of absolute cell volume can be obtained by hematocrit methods. Thus, Price-Jones[11] considers that the tightness of packing of the cells cannot be assumed to be uniform from one lot to another. In reply to these objections it may be said that uniformity of packing can be attained by centrifugalizing the cells until no further decrease in total cell volume takes place. This is usually attained by centrifugalizing the sample for fifteen and almost always for thirty minutes at 3000 revolutions per minute. Only occasionally is more time required for complete packing. If, after an initial centrifugalization for thirty minutes, readings are made at intervals of five minutes until no further change in volume of packed cells is observed, even this slight error can be eliminated.

It might be expected that by centrifugalization all fluid is forced out of the cell and only hemoglobin and plasma are left, thus making it impossible to obtain a true estimate of the size of the cells. Some experiments of Campbell[2] may be mentioned in this connection. This investigator mixed measured amounts of packed red cells with equal volumes of 0.6 per cent and 0.9 per cent saline solutions. The former swelled but hemolysis did not occur. After permitting equilibrium to take place between cells and saline, the specimens were stirred up and centrifugalized again. Repeated experiments showed that the cells in the weaker saline solutions remained proportionately larger than the cells in 0.9 per cent saline even after centrifugalization.

Jorgensen and Warburg[3] considered that the volume of the blood corpuscles is determined by osmotic forces so great that the force with which they are centrifugalized down is negligible compared with them. They add, further, that the volume of cells as determined by refractometric and viscosimetric methods agrees with the results determined by the hematocrit method.

TABLE 2

The Volume and Hemoglobin Content of the Erythrocyte; Fifty Normal Young Women

SUBJECT	AGE	CORPUSCULAR VOLUME	CORPUSCULAR HEMOGLOBIN	CORPUSCULAR HEMOGLOBIN CONCENTRATION
		cu. microns	γγ	per cent
1	30	70.9	27.0	38.8
2	28	80.8	27.4	34.0
3	18	76.6	27.4	35.8
4	20	74.2	27.9	37.6
5	17	77.6	30.0	38.7
6	21	76.6	26.4	34.5
7	18	78.0	30.4	39.0
8	24	82.9	29.0	35.0
9	20	73.7	27.0	36.7
10	20	75.1	27.0	35.9
11	19	84.2	28.8	34.1
12	20	76.5	27.0	35.2
13	19	75.3	26.8	35.6
14	20	79.4	25.8	32.6
15	19	73.8	26.6	36.1
16	20	85.7	27.1	37.3
17	20	81.6	28.8	35.4
18	20	80.0	26.3	32.9
19	18	81.3	27.8	33.6
20	23	82.9	30.4	36.6
21	20	82.1	28.0	34.4
22	21	77.8	27.5	35.4
23	18	79.8	26.0	37.6
24	19	83.5	27.6	33.0
25	19	82.4	25.4	30.9
26	21	81.8	27.2	33.2
27	20	80.1	28.2	35.2
28	22	80.0	29.8	37.2
29	23	83.5	29.4	35.3
30	22	83.9	29.8	35.3
31	21	80.5	27.8	34.7
32	21	82.3	29.8	36.2
33	20	80.8	29.9	34.9
34	22	79.0	28.8	36.4
35	30	79.5	28.2	35.5
36	18	81.9	27.8	33.9
37	17	81.9	27.8	34.1
38	25	79.0	25.4	32.3
39	28	77.3	28.4	36.8
40	22	83.1	30.9	37.2

TABLE 2—*Concluded*

SUBJECT	AGE	CORPUSCULAR VOLUME	CORPUSCULAR HEMOGLOBIN	CORPUSCULAR HEMOGLOBIN CONCENTRATION
		cu. microns	γ γ	per cent
41	30	81.6	28.4	34.8
42	19	84.8	27.4	37.3
43	30	84.3	29.0	34.5
44	20	81.4	28.0	34.1
45	19	80.8	27.6	34.2
46	20	85.0	28.8	33.9
47	19	82.9	29.0	34.9
48	23	70.6	23.2	31.4
49	24	84.7	29.4	34.8
50	27	79.1	27.7	35.1
Averages		80.1	28.0	35.2

Normal values for the volume and hemoglobin content of the average erythrocyte calculated from blood determinations in 100 healthy men residing in the southern United States were presented in an earlier report.[14] In table 2 values derived from accurate blood determinations in fifty healthy young women, seventeen to thirty years of age residing in the South are recorded[17]. Figure 1 is a histogram showing the frequency distribution of corpuscular volumes in the fifty normal women. The mean (80.1 cu.μ ±0.3) and the median (80.7 cu.μ ±0.3) are practically equal and coincide with the peak of the curve. The standard deviation is 3.9 cu.μ ±0.2 and the coefficient of variation 4.9 per cent. The minimal and maximal values were 70.6 cu.μ and 85.7 cu.μ. These, however, are probably not a true index of the variation, extremes being usually the result of errors in technic or due to imperfection in the sample. Standard deviation is a much better measure of variation. Eighty-four per cent of the observed corpuscular volumes ranged between 76 cu.μ and 84 cu.μ which are the values one standard deviation on each side of the mean.

In table 3, corpuscular volumes in men and women are compared. It is interesting to find that the values in the two sexes are almost identical. Calculations based on blood determinations reported by other investigators show an almost equal similarity in the values for both sexes.

In Table 4 are found average, maximal and minimal values for corpuscular volume as calculated from the available reliable data reported by other investigators for normal young women seventeen to thirty years of age. It will be noted that the values calculated from blood determinations by Osgood and Haskins in Oregon, and Haden in Missouri are distinctly higher than those
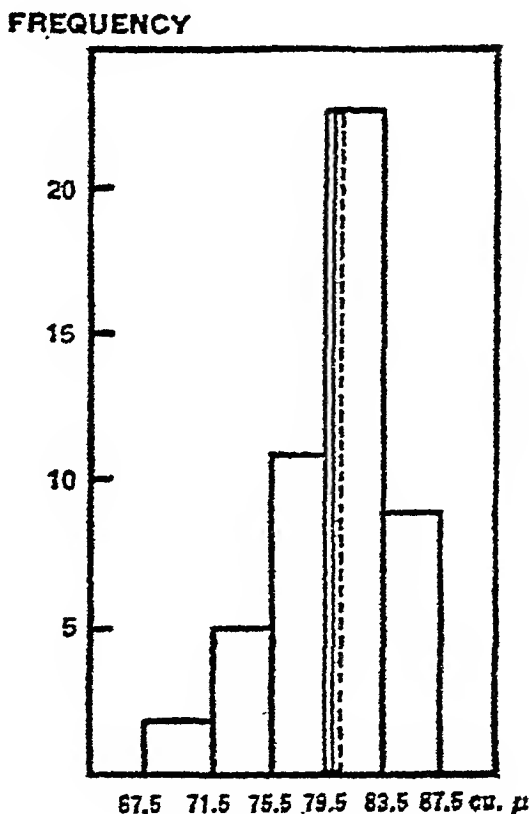
FREQUENCY

FIG. 1. HISTOGRAM SHOWING FREQUENCY DISTRIBUTION OF VARIATION IN CORPUSCULAR VOLUME IN 50 YOUNG WOMEN

The mean is indicated by a straight line, the median by an interrupted line

found in Louisiana. A similar difference was noted in the values calculated for men.[14] The cause of this difference is somewhat conjectural. The technic followed by Osgood and by me was practically the same and in each series solid potassium oxalate was used as the anticoagulant for the hematocrit determinations. Haden employed 1.6 per cent sodium oxalate solution.

Haden[7] recently reported his investigations concerning the effect of various anticoagulants on cell volume. His experiments indicate that the employment of potassium oxalate, either in solid form or in a saturated solution, causes more shrinkage in cell volume than has been allowed for by either Osgood[9] or by me[13,17]. Osgood found that 20 mgm. of oxalate added to 10 cc. of blood caused a shrinkage of 3.5 per cent, while I allowed 3.7 per cent for

TABLE 3

CORPUSCULAR VOLUME VALUES FOR PERSONS IN LOUISIANA

(In cubic microns)

	NUMBER	MEAN	MEDIAN	STANDARD DEVIATION,	COEFFICIENT OF VARIATION
					per cent
Men......	100	79.84±0.49	80.64±0.62	7.24±0.35	9.05
Women...	50	80 05±0.26	80.70±0.33	3.89±0.19	4.90

TABLE 4

VOLUME OF RED BLOOD CORPUSCLES IN 175 HEALTHY YOUNG WOMEN

(Expressed in cubic microns)

AUTHOR	NUMBER OF SUBJECTS	AVERAGE	MAXIMUM	MINIMUM
Osgood and Haskins (Oregon)[10].........	100	88.5	95.0	73.9
Haden (Missouri)[4]......................	9	92.8	98.1	90.5
Gram and Norgård (Denmark)[5]........	6	88.0	91.3	85.1
Bie and Möller (Denmark)[1]............	10	81.7	85.8	77.9
Wintrobe (Louisiana).......	50	80.1	85 7	70 6
Averages........................		85.9	91.8	74.4

shrinkage when oxalate was added in this proportion, and 6.7 per cent when 40 mgm. of oxalate were added to 10 cc. of blood.

I have since repeated my experiments and in sixteen determinations found that potassium oxalate used in the proportion of 10 mgm. to 5 cc. of blood caused on the average, a shrinkage of 5.75 per cent in the volume of packed erythrocytes as compared with the volume found in heparinized blood. The amount of shrinkage was not the same for each specimen of blood, nor was

this variation in amount of shrinkage altogether correlated with the relative amount of blood plasma and the consequent variation in dilution.

The difference between the original correction for shrinkage and the correction which these subsequent experiments indicate should be made, is not of sufficient magnitude to account for the differences in the values for mean corpuscular volume in the three localities mentioned. Besides, there is an appreciable difference between the values derived from Osgood's blood determinations and those derived from my own investigations, although the amount of correction for shrinkage was in each case practically the same. It is just possible, then, that the differences in corpuscular volume are dependent on other factors than differences in technic[4]. It is hoped that further determinations will be made in other localities.

In summing up it may be said that, on the basis of the blood determinations presented in this and in an earlier paper[14], the normal range of corpuscular volume in healthy young men and women residing in the Southern United States may be considered to be 75 cu.μ to 92 cu.μ, while the normal range for all localities is about 75 cu.μ to 95 cu.μ with an average of 85 cu.μ. Values above 95 cu.μ or below 75 cu.μ are probably a manifestation of abnormality.*

MEAN CORPUSCULAR HEMOGLOBIN

Mean corpuscular hemoglobin—the amount by weight of hemoglobin in the average red corpuscle—may be calculated by dividing the amount of hemoglobin, expressed in grams per 1000 cc. of blood, by the number of erythrocytes expresses as millions

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* It is of interest to note that Ponder and Saslow have recently reported (Jour. Physiol., 70: 18–37. 1930) the average corpuscular volume, as determined by an accurate and laborious colorimetric method in ten individuals, to be 87.1 cu.μ. This corresponds closely to the mean value presented in this paper (85 cu.μ) and, contrary to the expressed opinion of Ponder and Saslow, suggests the adequacy of the hematocrit method for the determination of erythrocyte volume.

per cubic millimeter. The resulting value expresses mean corpuscular hemoglobin in micromicrograms.*

Calculations based on blood determinations in one hundred healthy young men residing in the South showed an average corpuscular hemoglobin of 29.2γγ[14]. Average corpuscular hemoglobin based on 274 accurate blood determinations in different parts of the world was likewise 29.2γγ. Figure 2 shows the frequency distribution of corpuscular hemoglobin values based
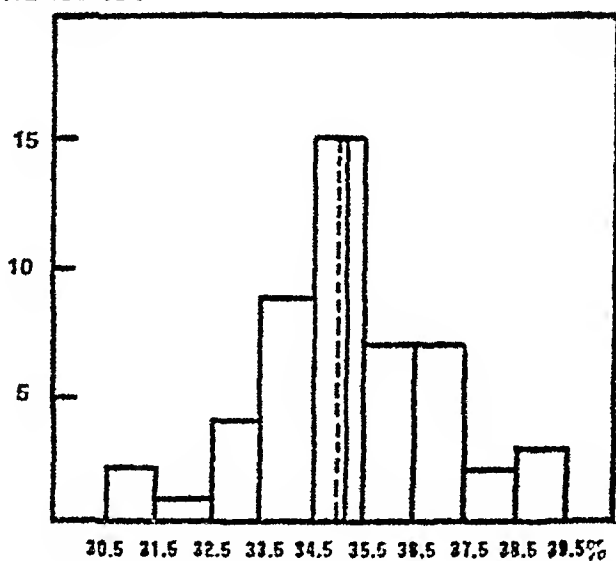
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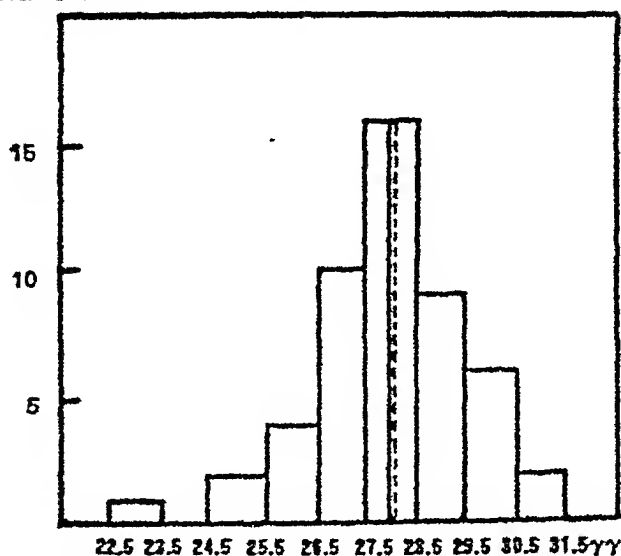
FIG. 2. HISTOGRAM SHOWING FREQUENCY DISTRIBUTION OF VARIATION IN CORPUSCULAR HEMOGLOBIN IN 50 YOUNG WOMEN

The mean is indicated by a straight line, the median by an interrupted line

on determinations in fifty healthy young women, seventeen to thirty years of age, residing in the Southern States. The mean (27.96γγ) and the median (28.00γγ), which are practically identical, coincide with the peak. The standard deviation is 1.55γγ and the coefficient of variation is 5.54 per cent. Measuring by means of the standard deviation, the significant variation is 26.5 to 29.5γγ. Actually 86 per cent ranged between these values.

* A micromicrogram is the millionth of a millionth part of a gram, or gram × 10⁻¹². It is abbreviated by the Greek letters γγ.

In table 5 corpuscular hemoglobin values in the men and women examined in Louisiana are compared. These further determinations support the conclusions reached in an earlier report,[14] namely, that there is no appreciable variation of corpuscular hemoglobin in respect to sex.

In table 6 corpuscular hemoglobin values calculated from data available for 175 healthy women seventeen to thirty years of age,

TABLE 5

CORPUSCULAR HEMOGLOBIN VALUES FOR MEN AND WOMEN IN LOUISIANA

(Expressed in micromicrograms)

	NUMBER	MEAN	MEDIAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
					per cent
Men......	100	29.18±0.17	29.89±0.21	2.51±0.12	9.18
Women...	50	27.96±0.15	28.00±0.18	1.55±0.10	5.54

TABLE 6

CORPUSCULAR HEMOGLOBIN VALUES FOR HEALTHY YOUNG WOMEN

(Expressed in micromicrograms)

AUTHOR	NUMBER OF WOMEN	AVERAGE	MAXIMUM	MINIMUM
Osgood and Haskins[19]..................	100	28.5	32.4	23.7
Haden[6]............................	9	31.3	31.8	30.0
Gram and Norgäard[5]..................	6	27.9	28.9	27.1
Bie and Möller[1].....................	10	28.1		
Wintrobe...........................	50	28.0	30.9	25.4
Averages...........................		28.4	31.8	24.7

residing in different parts of the world, are given. The average of these corresponds closely with the average in 274 healthy men. There appears to be no great variation in corpuscular hemoglobin in the different localities considered. On the basis of the data at present available it can be said, then, that the normal corpuscular hemoglobin in the young adult is 28 or $29\gamma\gamma$, while the range of normal is 26.5 to $31.5\gamma\gamma$.

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION

The hemoglobin concentration of the average red corpuscle may be calculated by dividing the amount of hemoglobin, expressed in grams per 100 cc. of blood, by the volume of packed erythrocytes, expressed as cubic centimeters per 100 cc. of blood. The resulting value, multiplied by 100, expresses the mean corpuscular hemoglobin concentration in per cent.

FREQUENCY

FIG. 3. HISTOGRAM SHOWING FREQUENCY DISTRIBUTION OF VARIATION IN CORPUSCULAR HEMOGLOBIN CONCENTRATION IN 50 YOUNG WOMEN

The mean is indicated by a straight line, the median by an interrupted line

In view of the fact that the manner of distribution of hemoglobin in the red cell is still unsettled, the term "concentration," implying as it does a state of solution, is used with some hesitation. However, since the relationship here considered is one of weight to volume, namely the amount of hemoglobin in proportion to the volume of the erythrocyte, the term "corpuscular hemoglobin concentration" or, more briefly, "corpuscular concentration" is used to express this relationship on the excuse of being descriptive and convenient and not with any implication as to the manner of distribution of the hemoglobin.

The average corpuscular concentration based on blood determinations in one hundred healthy young men in Louisiana, was 36.6 per cent, whereas the average of calculations based on 231 determinations on men of the same age group in different parts of the world was 34.6 per cent[14]. Figure 3 shows the frequency distribution of corpuscular concentration values based on blood determinations in fifty healthy women seventeen to thirty years of age, residing in the South. The mean (35.2 per cent) and the

TABLE 7

CORPUSCULAR HEMOGLOBIN CONCENTRATION VALUES FOR MEN AND WOMEN IN LOUISIANA

	NUMBER OF SUBJECTS	MEAN	MEDIAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
					per cent
Men......	100	36.6±0.2	36.2±0.3	3.0±0.1	8.6
Women...	50	35.2±0.2	35.1±0.2	1.8±0.1	5.2

TABLE 8

CORPUSCULAR HEMOGLOBIN CONCENTRATION VALUES FOR NORMAL YOUNG WOMEN

AUTHOR	NUMBER OF SUBJECTS	AVERAGE	MAXIMUM	MINIMUM
Osgood and Haskins[10].....................	100	32.2	33.7	29.0
Haden[6]............................	9	33.7	34.4	32.2
Gram and Norgäard[5].....................	6	31.7	34.1	30.9
Bie and Möller[1]......................	10	34.4		
Wintrobe............................	50	35.2	39.0	31.4
Averages............................		33.3	35.4	30.0

median (35.1 per cent) coincide with the peak. The significant variation on the basis of the standard deviation of 1.8 per cent, is 33.4 to 37.0 per cent. Actually 80 per cent ranged between these values.

In table 7 corpuscular concentration values in the men and women examined in Louisiana are compared. As was the case with corpuscular volume and corpuscular hemoglobin, corpuscular concentration shows no significant variation in respect to sex.

Corpuscular concentration values based on determinations made on 175 young women in different parts of the world are shown in table 8. The average of these values corresponds closely with the average in 231 healthy young men. From the data at present available it appears, then, that the average hemoglobin concentration of the red cell in the normal young adult is 35 per cent while the range of normal is approximately 33 to 39 per cent.

ILLUSTRATION OF METHOD OF CALCULATION

A sample of blood contains 5.5 million red blood cells per cubic millimeter, 15.5 grams of hemoglobin per 100 cc. of blood, and 45.2 cc. of packed red cells per 100 cc. of blood. Then,

Mean corpuscular volume is $\dfrac{452}{5.5} = 82.2$ cu. μ

Mean corpuscular hemoglobin is $\dfrac{155}{5.5} = 28.2 \gamma\gamma$

Mean corpuscular hemoglobin concentration is $\dfrac{15.5}{45.2} \times 100 = 34.3$ per cent

It is not within the scope of this paper to present the details of corpuscular volume, corpuscular hemoglobin and corpuscular concentration determinations in the various types of anemia. It may be mentioned, however, that I have found these calculations of great value in affording a clearer conception of the alterations in the physical state of the erythrocytes associated with various diseases. In the diagnosis of pernicious anemia and sprue corpuscular volume determinations have proved to be of particular value. Although mean cell diameter is not always significantly greater than normal in these diseases[12], I have found mean corpuscular volume always distinctly greater than normal during the stages of relapse. Differences in the size of erythrocytes are probably present in all dimensions. Thus differences which may be insignificant when only one dimension such as diameter is measured, are readily noted when corpuscular volume is calculated. Although it might be expected that the small and distorted cells found in the blood of patients suffering from perni-

cious anemia would tend to lower the mean volume of the cells, actually the effects of even marked anisocytosis and poikilocytosis have not been found to be sufficient to reduce mean corpuscular volumes below values which are significantly higher than normal.

Finally it may be mentioned that a most interesting feature of blood determinations in a large series of anemias of all types is the possibility that all anemias may be subdivided into four distinct classes on the basis of differences in the size and hemoglobin content of the erythrocytes.[19]

SUMMARY

The inaccuracy and inadequacy of hematologic technic is pointed out and the vagueness and inaccuracy of the color and other indices discussed. For reasons of simplicity, increased clarity and greater accuracy, the direct calculation of the average volume of the red blood corpuscle, the amount of hemoglobin it contains, and the "concentration" of hemoglobin in the cell is proposed. Simple methods for such calculations are presented.

Normal values for corpuscular hemoglobin, corpuscular volume and corpuscular hemoglobin concentration, calculated from accurate blood determinations carried out in different parts of the world, are given. These may be summarized as follows:

1. The quantity of hemoglobin in the average erythrocyte (mean corpuscular hemoglobin) of the blood of a healthy young adult is 28 to 29 micromicrograms. The range of normal is 26.5 to 31.5$\gamma\gamma$.

2. The volume of the average erythrocyte (mean corpuscular volume) of the blood of a healthy young adult residing in the southern United States is 75 cu.μ to 92 cu.μ. In other localities for which blood determinations are available, the average corpuscular volume appears to be somewhat greater than this. On the basis of accurate data at present available the extreme variation of corpuscular volume in all localities may be considered as being 75 to 95 cu.μ, while the average corpuscular volume may be taken to be 85 cu.μ.

3. The proportion of hemoglobin in the average erythrocyte (mean corpuscular hemoglobin concentration) of the blood of a healthy young adult is 33 to 39 per cent and averages 35 per cent.

No differences in corpuscular volume, corpuscular hemoglobin, or corpuscular hemoglobin concentration have been observed in respect to sex.

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GASTRIC MANIFESTATIONS OF LYMPHATIC ALEUKEMIA (PSEUDOLEUKEMIA GASTRO-INTESTINALIS)*

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Development of major clinical symptoms in association with extensive infiltration of the stomach in various forms of lymphomatous disease (lymphomatosis) is considered uncommon. A presumptive diagnosis may be ventured by biopsy of the local mass or of the peripheral node at operation. Without such biopsy and in the absence of a palpable spleen or peripheral masses or of a leukemic blood picture, clinical diagnosis may become well nigh impossible. Gastric deformities reproduced on the roentgenograms may easily be interpreted as due to cancer.

REPORT OF CASE

A male, seventy-one years of age, a mild diabetic for about twenty years, obese and comparatively healthy, complained of a continuous dull pain in stomach, in November, 1921. This had been present for many years and was relieved only partially by soda. In January, 1923, the condition became acutely aggravated. Self-induced vomiting relieved the distress. The vomitus contained particles of food ingested two days before but gastric lavage sometimes failed to recover raisins eaten the night before. Gastric analysis showed free hydrochloric acid 38° and total acidity 54°. Stools were negative for occult blood. There was indefinite soreness over the epigastrium but a mass could not be palpated. Roentgen examination showed a slightly enlarged, hypotonic stomach with a retention of barium for twenty-eight hours. The pylorus could not be made to fill properly and the duodenal cap was poorly made out. A beginning carcinoma or a concealed ulcer, at the pylorus, with retention was considered a possibility, both clinically and roentgenologically. No definite di-

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agnosis was reached at the time, however, and the patient continued to be treated symptomatically with indifferent results. More or less gastric distress was present every day, especially after meals. December 1, 1923, the patient developed acute epigastric pain with vomiting and unusual amounts of gas. Roentgen examination at this time showed an enlarged stomach with sluggish peristalsis and spastic deformity at the pylorus. The duodenal cap showed a definite filling defect. A small amount of barium residue was noted at the end of forty-eight hours. Roentgen diagnosis of chronic duodenal ulcer with acute retention was made. Laporatomy was performed. A large mass about the size of an orange obstructing the orifice was found in the region of the pylorus. The regional lymph nodes were enormously enlarged, quite hard on palpation and extensively involved. The head of the pancreas was also involved in the mass. The liver showed no evidence of metastasis. A gastro-enterostomy was performed. Diagnosis of probable carcinoma of the stomach was made without biopsy. Before operation, the hemoglobin was 92 per cent, erythrocyte count, 5,400,000 and leukocyte count, 18,300. The latter was 7,250 two weeks later. Three weeks later, a second laporatomy was performed to relieve intestinal obstruction due to adhesions. The gastric tumor was noted to be considerably reduced in size at this time.

Subsequent history was like that of an usual mild diabetic whose urine sugar was controlled by diet and whose blood sugar was never over 0.285 per cent. The patient was well built, rather obese, weighing about 78 kilograms. He presented himself, from time to time, for various minor ailments such as chronic urethritis, bilateral otitis media, impairment of vision due to hyaline degenerative spots in the left macula, et cetera.

In December, 1928, the patient for the first time complained of intermittent heart beats. Electrocardiograms showed left and right ventricular extrasystoles. Blood pressure was 110 mm. systolic and 55 mm. diastolic and pulse 58 per minute. The cardiac condition became progressively worse and in April, 1929, edema of both legs and irregularity of pulse developed. In August, 1929, the enlargement of axillary and inguinal lymph nodes was noticed for the first time. In September, 1929, hemoglobin was 70 per cent, erythrocyte count, 4,010,000 and leukocyte count, 15,600 with a differential count showing neutrophiles, 49 per cent, lymphocytes, 47 per cent, monocytes, 3 per cent and eo-inophiles, 1 per cent.

On January 31, 1930, swelling of the right abdomen and swelling and pain in the left leg developed. Regional glands increased in size and number, some in the axilla having reached the size of a hen's egg. A few nodes, discrete, movable and fairly soft in consistence, were palpable in the neck. The hemoglobin was 45 per cent, erythrocyte count, 3,080,000, leukocyte count, 18,500 with a differential count showing 54 per cent neutrophiles, 43 per cent lymphocytes and 3 per cent monocytes. No pathological forms of lymphocytes were noted in the study of the blood smears. Biopsy of an axillary node was done on February 4

to determine whether the cause of the adenopathy was due to Hodgkin's disease, leukemic infiltration or metastatic carcinoma.   Histologic diagnosis was leukemic infiltration of the lymph node.

On February 10, 1930, the patient died of circulatory failure.

The body was that of a well developed, obese, adult, white male 170 cm. long and approximately 82 kgm. in weight.   The body was embalmed and several trochar punctures were present in the abdominal wall.   The skin and the mucous membrane showed a moderate degree of palor.   A long surgical scar 15 cm. in length was noted in the upper abdominal wall to the right of the mid-line.   The cervical nodes were palpable, those on the left anterior triangle were quite prominent.   The axillary nodes were swollen and protruded prominently on both sides along the anterior axillary line, some approximating 6 cm. in diameter. The inguinal nodes were likewise enlarged.   They were discrete, well encapsulated and soft.   The cut surface was pinkish-white, homogeneous and rubbery in consistence.   The subcutaneous fat was 2 cm. in thickness.   The subcutaneous tissue was swollen and edematous.   The scrotum and the penis were greatly edematous.   No jaundice, cyanosis or hypostasis was noted.

Incision was limited to the upper abdomen.

The peritoneal cavity contained a large amount of fluid mixed with embalming liquid.   The appendix was 10 cm. long and 1 cm. in average diameter.   It was firm, solid (figs. 3a, b) and easily broken.   The cut surface consisted of a solid, homogeneous, white, cellular tissue surrounded by a thin muscular layer. The liver border extended 2 cm. from the costal margin.

Each pleural cavity contained a large amount of clear, amber fluid, estimated at 1500 cc.   Both lungs were collapsed against the spinal column.

The heart and the lungs were not removed for examination.   Manual examination revealed a slightly enlarged heart with a definite hypertrophy of the left ventricle which was firmly contracted.

The lungs were both collapsed and shrunken.   They showed no areas of consolidation.

The spleen weighed approximately 120 grams.   The capsule was grayish and slightly thickened.   The cut surface showed a dark red, soft pulp.   The corpuscles were numerous and quite prominent, ranging in size from 1 to 3 mm. in diameter and especially numerous beneath the capsule.

The liver appeared about normal in size.   It was pale, grayish and soft. The cut surface was swollen, cloudy and showed a pale yellowish-brown parenchyma, regularly studded with hemorrhagic spots which surrounded the lobular centers.

The stomach was definitely enlarged weighing approximately 750 grams and measuring 46 cm. from the cardia to the pyloric ring and 25 cm. in greatest circumference.   The serosa was thickened and fibrous.   Many enlarged lymph

nodes were adherent along both the lesser and the greater curvatures. The entire wall was tough and leathery in consistence (fig. 1). The mucosa was enormously hypertrophied (fig. 2) and on section, white and diffusely cellular and up to 1.5 cm. in thickness. The rugae along the cardiac half of the stomach were in deep and thick folds, like the convolutions of the brain, up to 2 cm. in height and 2.5 cm. in thickness. In the distal half, the mucosa was velvety, showed a few wide, longitudinal furrows along the lesser curvature and a few, diffuse, ill-defined elevations. Proximal to the pyloric orifice was a round, tumor-like mass, 6 by 6 by 5 cm. which was found lying within the wall but
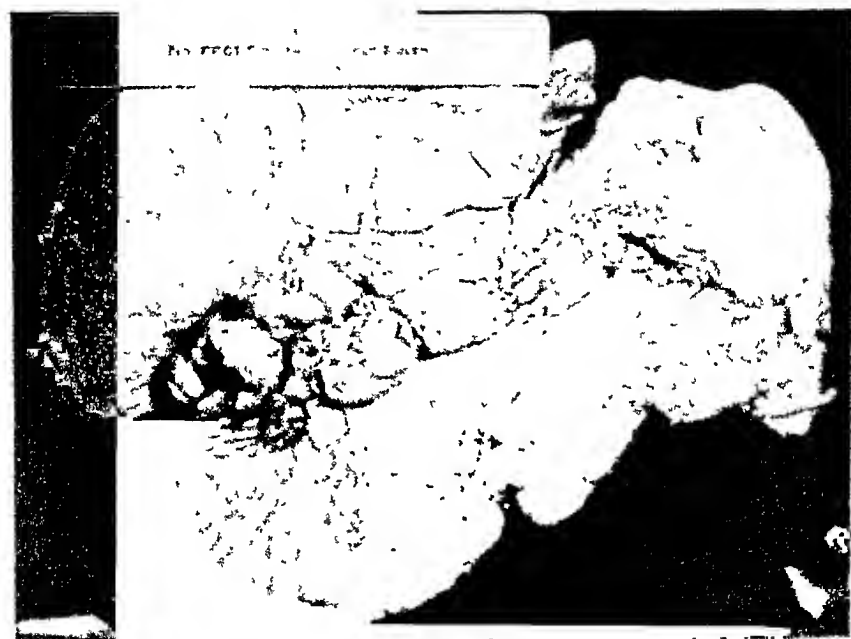
Fig. 1. Stomach, peritoneal surface. Thickened, fibrous, leathery serosa, several enlarged lymph nodes and a large lymphoma at the pylorus.

protruding into the pyloric antrum and infiltrating into the ring. The cut surface of this mass showed a soft, pinkish-gray, homogeneous lymphoid tissue. Along the dependent part of the greater curvature was a functioning gastro-enterostomy stoma.

The mucosa of the small intestine was edematous. The lymphoid follicles were conspicuous throughout. The Peyer's patches showed irregular hypertrophy and edema, stones up to 1 cm. in height. The colon was somewhat dilated. The wall was thinned. The mucosa was studded with many nodular lymphoid follicles up to 5 mm. in diameter and 3 mm. in height. At the cecum, there were two nodular tumors, one, 1 cm. in diameter and 3 cm. in height and the other,

a rounded, umbilicated elevation 3 cm. in diameter and 2 cm. in height, around the orifice of the ileocecal valve.   The mucosa was reddened and hemorrhagic while the surface of the mass was roughened, hemorrhagic and showed superficial gangrenous discoloration (fig. 4).

The head of the pancreas was replaced by a soft, white tumor-like tissue. The splenic vein was filled with a firm, organized coagulum.   Small irregular areas of white, soft tumor-like tissue were scattered in the substance of the pancrease.

The adrenal glands were normal.
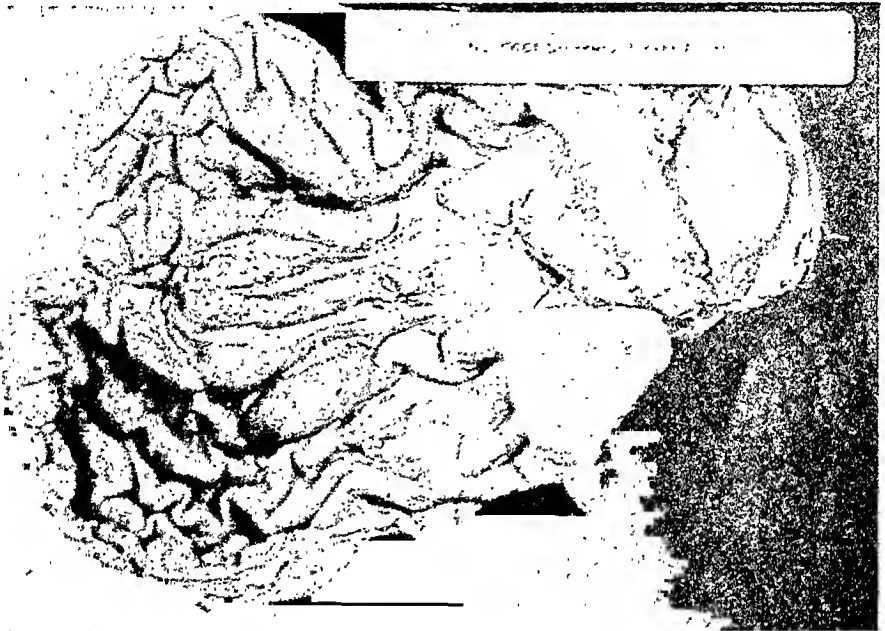
FIG. 2. Stomach, opened: Rugal folds resembling "the convolutions of the brain;" note thickness of the mucosa; note a mass at the pyloric end with narrowing of the lumen.

The right kidney weighed 160 grams.   The capsule was grayish, about 2 mm. in thickness and adherent.   It stripped with some difficulty and left a whitish, roughened, mottled surface.   The cut surface revealed a grayish parenchyma peppered with punctate hemorrhagic areas.   The surface was swollen and markings were rather indistinct.   The left kidney with the capsule was 15 by 8.5 by 8.5 cm. and weighed 700 grams.   The capsule was fibrous, leathery, 4 mm. in thickness and apparently infiltrated by tumor cells.   It was firmly adherent to the cortical surface which was grayish, roughened and covered with residual adhesions.   The cut surface was waxy and swollen.   The cortex was 1.5 cm. in width and mottled with many punctate hemorrhages and markedly

infiltrated with grayish tumor-like tissue. The pyramids were dark red and stood out conspicuously against a background of a whitish, waxy parenchyma. The mucosa of the pelvis was swollen and hemorrhagic.

The mesenteric, retroperitoneal and regional lymph nodes along the stomach, pancreas, hilum of the liver as well as the cervical, axillary and inguinal nodes were enormously increased in number and showed enlargement varying in diameter from 1 cm. to 6 cm. They were discrete but found in conglomerate masses. They were pinkish-gray in color, uniformly soft, friable and appeared quite cellular. Involvement of the posterior gastric and peripancreatic nodes was most extensive.
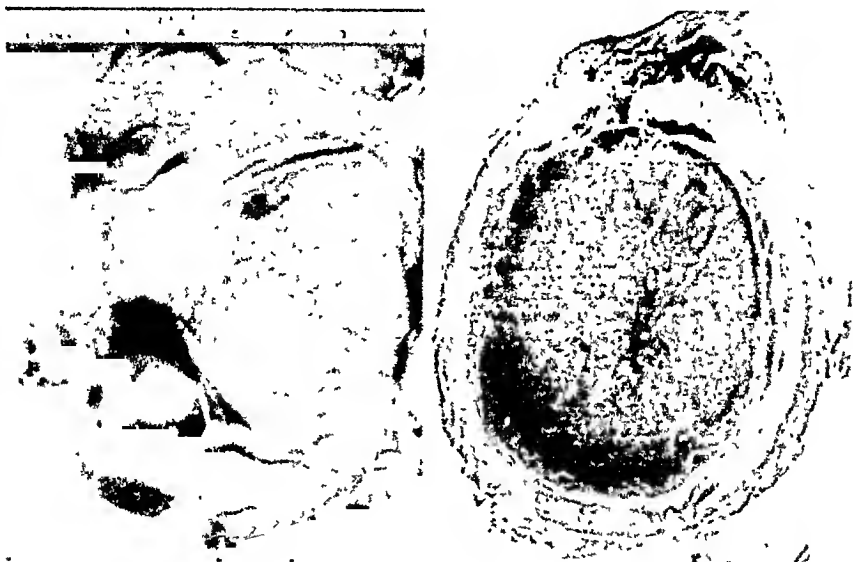
FIG. 3a. Cecum, peritoneal aspect: Appendix buried in adhesions; note a cross-section of the appendix; several enlarged nodes.

FIG. 3b. Appendix showing a massive leukemic growth obliterating the lumen and replacing the entire mucosa and submucosa; infiltrations into the muscularis and out into the serosa and meso-appendix.

Diagnosis (limited to the abdominal viscera)

1. Leukemic infiltration and lymphoma of the stomach and intestines.
2. Leukemic lymph nodes (mesenteric, retroperitoneal, perigastric, pancreatic, cervical, axillary and inguinal).
3. Leukemic infiltration of the kidneys and the pancreas.
4. Chronic passive congestion and cloudy swelling of the liver.
5. Gastro-enterostomy.
6. Thrombosis of the splenic vein.
7. Bilateral hydrothoraces and ascites.

Microscopic study

Stomach: The mucosa of the stomach showed areas of superficial necrosis over the periphery. Beneath, the glandular architecture was completely obliterated by diffuse invasion of lymphocytes which freely infiltrated into the submocosa (fig. 5) and aggregated into many pseudofollicular masses (fig. 6) up to 1 mm. in diameter. The muscularis was diffusely hypertrophied. The lymphocytes infiltered deeply into the muscle wall and out into the thickened

Fig. 4. Cecum, opened: Two large lymphomatous masses: numerous small lymphoid nodules.

serosa, forming multiple nests of cells in scattered areas. The cells were uniform in size and shape, of the undoubted adult lymphocyte type, showing a large round nucleus with coarse hyperchromatic strands of chromatin and showed no mitosis (fig. 7). This, essentially, represented a sectional view of an average ruga.

The tumor-like mass at the pylorus showed a histologic structure identical with that seen in the lymph nodes. It was a diffuse mass of the lymphocytes supported by a scanty stroma carrying a few fine capillaries. A few large, pale

Fig. 5   High power picture of the gastric mucosa

Fig. 6   Low power section of a mucosal fold of the stomach, role many pseudofollicles immediately beneath the glands and diffuse hyperplasia of lymph nodes and invasion into the muscularis

staining cells of the reticulo-endothelial type might be seen through the mass of the more dark staining lymphocytes. No mitotic figures were observed. Neither were there any multi-nucleated giant cells of the Dorothy Reed type.

Lymphoid hyperplasias and infiltrations of the identical character were noted in the terminal ileum and the cecum.

The pancreas showed diffuse interlobular masses of lymphocytes which infiltrated also between acini. Interstitial fibrosis and hyaline degeneration of the islands of Langerhans were also noted.

The kidney showed the most striking picture of leukemic infiltration through-
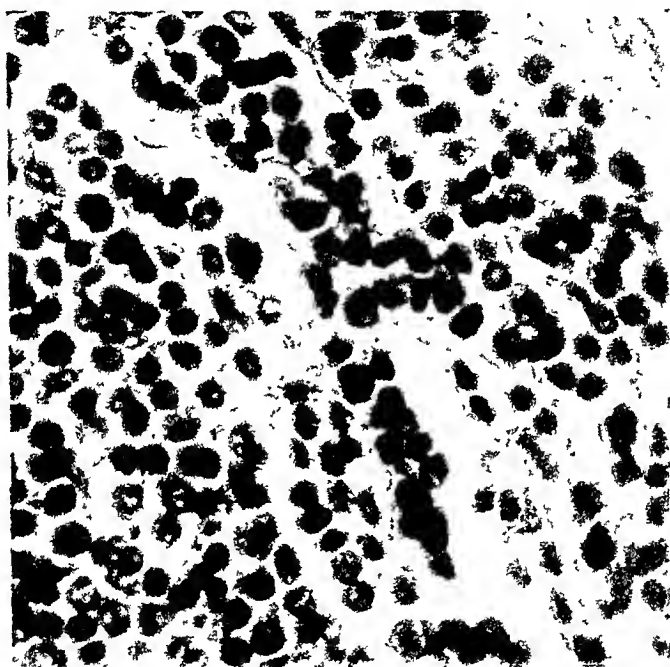
Fig. 7. A higher power view of the lymphocytes; note the uniformity of size, shape, staining quality, et cetera.

out the cortex and deep in the medulla, the lymphocytes spreading in large masses and cords.

The liver and the spleen showed the minimal perivascular accumulation of lymphoid cells. Diffuse hemorrhage at the lobular centers with atrophy of the hepatic cords.

Microscopic diagnosis

1. Leukemic infiltration of the stomach, intestines, spleen, liver, kidneys, pancreas and lymphatic nodes.
2. Chronic passive congestion of the liver.
3. Hyaline degeneration of the islands of Langerhans of the pancreas.

COMMENT ON THE CASE

In the light of the necropsy, the following observations may reasonably be drawn:

The gastric symptoms must be attributed to the leukemic changes in the wall of the stomach which consisted essentially of the diffuse leukemic infiltration of the mucosa and the localized lymphomatous growth at the pylorus. It is reasonable to conclude that these symptoms had been present, at least, since early in 1921 or nine years prior to death and that the roentgen findings in the stomach in 1923 were due to these leukemic changes. The pyloric tumor and the enlarged regional lymph nodes found at operation were leukemic masses and not cancerous growth and regional metastases, as clinically diagnosed. In the absence of a leukemic blood picture and of demonstrable peripheral adenopathy, a correct diagnosis was clinically impossible at the time. Without biopsy at the time of laporatomy, a diagnosis of probable carcinoma of the stomach seemed reasonable. Indeed, the clinical and roentgen observations as well as the operative findings were strikingly suggestive of gastric cancer. A five-year cure might have been thus recorded, illustrating again the vital importance of biopsy, if at all possible, in even the most obvious cases of clinical cancer.

Noteworthy also is the fact that advanced as was the involvement of the lymph nodes in and about the wall of the stomach and the pancreas at the time of laporatomy in 1923, peripheral adenopathy was not noted until five years later, a clinical observation which, while not uncommon in leukemias and aleukemias, nevertheless, deprived a possible means of early diagnosis in this case. When the peripheral nodes finally became demonstrable and a biopsy diagnosis of lymphatic aleukemia was made, it was considered rather as an independent clinical finding than either as an etiologic factor of the existing cardiac lesion or as the cause of the gastric symptoms which had long subsided following the gastro-enterostomy seven years before.

GENERAL DISCUSSION

The involvement of the stomach in various forms of the so-called lymphomatosis is rare in routine experience although it is

not infrequently reported in literature. When encountered at necropsy, unless some guiding clinical data are at hand, the exact anatomical diagnosis may be difficult, while the histological picture may be equally confusing. Thus, Hodgkin's granuloma, lymphosarcoma, leukosarcoma, leukemia and pseudoleukemia, all may similarly involve the stomach. With minor variations, they may present an identical general gross appearance. This may be represented by a localized thickening or induration of the wall or one or more circumscribed tumors and nodules, with or without ulceration. Sometimes this condition is characterized by a diffuse thickening of the wall with prominent, deep, sinuous folds of the thickened mucosa or rugae, usually described as resembling "the convolutions of the brain," with or without localized nodular elevations or by one or more circumscribed flat thickenings of the mucosa. The regional lymph nodes are invariably involved. Microscopically, the general granulomatous picture with areas of fibrosis in Hodgkin's disease, the invasive character in lymphosarcoma, the malignant behavior with a leukemic blood picture in the so-called leukosarcoma and the characteristic organ infiltration and blood picture in leukemia, may each determine a final diagnosis. The fact is, however, that a differential pathological diagnosis between these conditions, presenting as they do a similar anatomical and clinical picture and often a confusing microscopic behavior, is by no means simple but difficult at times.

Because of this similarity and of the apparently close etiological relationship which exists between these conditions, a certain school of pathologists prefer to group them all under a general term of lymphoblastoma[12] while others consider them as lymphosarcomas or malignant lymphomas. The final diagnosis is thus made a matter of individual training and conception, particularly when it is done solely on the anatomic and histologic basis without the clinical data including the blood picture. On the other hand, Simonds[20], concluding his recent review on Hodgkin's Disease, makes the following timely statement:

"The progress thus far made in the study of diseases of the lymph glands has been the result of the splitting up of the composite group of pathologic conditions originally described by Hodgkin, into leukemia, pseudoleukemia,

lymphosarcoma and Hodgkin's disease.   There is a tendency to recombine these diseases into a common group.   Until their etiology is discovered, the final solution of the problem of their relation to each other will be furthered by rigidly maintaining the histo-pathologic criteria already devised for their differentiation."

HODGKIN'S GRANULOMA AND LYMPHOSARCOMA OF THE STOMACH

Many cases of Hodgkin's granuloma involving only the stomach or the gastro-intestinal tract have been reported during the last two decades.

Schlagenhaufer,[19] Eberstadt,[6] Reimann[18] and Partich[16] made important contributions on this subject.   More recently, Hayden and Appelbach[11] reviewed twenty-six such cases with cardinal anatomic lesions practically entirely limited to the digestive tract. Microscopically, the representative lesion in these cases is typically granulomatous and as such, differentiation from the remaining groups of lymphomatous growths appears less difficult.

Of 592 sarcomas of the digestive tract collected by Goldstein[9] up to 1921, 265 occurred in the stomach.   According to various authorities, sarcoma of the round cell type constitutes by far the most predominant among the sarcomas of this organ and the majority of the so-called round cell sarcomas, most probably, belong to a group identified as lymphosarcoma.[13]   Indeed, in this group, are probably included an appreciable number of leukemic growths, pseudoleukemias as well as Hodgkin's granuloma of the more cellular type.[24]

LEUKEMIC INFILTRATION OF THE STOMACH

A characteristic attribute of leukemia is its tendency to infiltrate the visceral organs, notably the spleen, the liver, and the kidneys, as well as the lymphoid tissue everywhere, to a greater or less extent.   The degree of infiltration is a variable factor; one or more of these organs may show a greater or less involvement than others.   The blood itself may not be an exception, a normal count or a normal morphology of the leukocytic elements or both, persisting throughout life.   Localized lymphoid hyperplasias in the wall of the gastro-intestinal tract in leukemia, if carefully searched for at necropsy, are probably not uncommon, although

clinical symptoms arising from such are seldom encountered. Diffuse or massive leukemic infiltration of the gastric wall with the production of major symptoms there from is undoubtedly infrequent.   Leudet[14] described very curious lesions (infiltration) consisting of mucosal elevations and soft, round masses in the wall of the stomach at autopsy in a case of leukemia.   DeRoth[5] stated that when the stomach was affected in leukemia, there was a considerable thickening of the mucosa which became wrinkled and resembled the "convolutions of the brain."   Such a degree of involvement of the gastric wall, however, is quite uncommon in proven cases of leukemia.

Symmers[24] made this observation:

"In true lymphatic leukemia the lymphoid structures of the gastro-intestinal tract rarely, if indeed they ever, undergo a degree of hyperplasia, even remotely comparable to that just described (pseudoleukemia gastrointestinalis). In nineteen cases of chronic lymphatic leukemia of which I have records, the gastro-intestinal lymphoid structures were practically unchanged."

The archives in the Department of Pathology of the University of Minnesota reveal that, of 12,396 necropsies performed from 1900 to 1928, inclusive, there were seventy-seven cases of leukemia recorded, of which fifty-one cases were of the lymphatic type and twenty-six of the myelogenous type.   Of the fifty-one cases of lymphatic leukemia, two showed a stomach with a local nodular elevation or thickening and two ulcers.   Of the twenty-six of myelogenous leukemia, only one showed a local thickening.   The following clinical case[26] is cited as a pertinent example.

The patient, an elderly white man of sixty-two years was admitted to the University Hospital in June, 1929, complaining of gastric distress, with loss of weight, abdominal distension with visible peristalic waves and absence of free gastric hydrochloric acid, presenting a clinical picture of gastric cancer. His leukocyte count ranged from 13,000 to 40,000 per cubic millimeter with 90 per cent of lymphocytes, on the basis of which a diagnosis of chronic lymphatic leukemia was made. Roentgen study of the gastro-intestinal tract revealed an enormous thickening of the rugal folds throughout the entire mucosa of the stomach and first portion of the duodenum. A roentgen diagnosis of probable chronic hypertrophic gastritis or multiple polyposis with malignant degeneration was made, and an exploratory laporatomy was performed. The stomach was enormously dilated. The wall was diffusely thickened and nodular. There

were many enlarged lymph nodes along the greater curvature. Thickening extended all along the duodenum and the small intestine to the cecum where large masses were palpated within the lumen. Biopsy of a regional lymph node showed leukemic hyperplasia.

Sternberg[21] recognized an intermediary form between the true lymphosarcomas and the leukemias and designated it as "leukosarcoma." Flashman and Leopold[5] reported a case of this kind in which a primary lymphoma was found in the pelvis without a leukemic blood picture. Following intensive roentgenotherapy with disappearance of the mass and temporary clinical improvement, the patient returned with a rapidly progressive leukemic blood. At autopsy, the stomach showed an enormous thickening of the mucosa with a typical configuration likened to the brain convolutions. Warthin[27] advocating the theory of the neoplastic nature of leukemia, reported a case in a man of thirty years, suffering from jaundice, constipation and gastric symptoms, in whom "between 80 and 90 per cent of the 90,000 white cells were atypical mononuclears" and who died after operation. At autopsy, the stomach as well as the small intestine showed a diffuse growth, in general resembling a lymphosarcoma. The growth was fairly vascular and the cells showed a marked tendency to invade the vessels. In the stomach, the tumor cells could be seen infiltrating the muscle. Warthin advanced a name "leukoblastoma" for this type of lymphatic manifestation.

"PSEUDOLEUKEMIA GASTRO-INTESTINALIS"

Conheim[4] recognised anatomic characteristics in leukemia without changes in the blood and suggested the name pseudoleukemia. Cabot[13] defined it "as a hyperplasia of specially hemopoietic tissue closely akin to leukemia, in fact, distinguished therefrom solely by the absence of leukemic changes in the peripherial blood." Sternberg[22] emphasized distinct features of pseudoleukemia and separated it as an entity from Hodgkin's disease. Ewing[7] considers "true pseudoleukemia" as a systemic aleukemic lymphomatosis while Warthin[11] designates the condition as a generalized (or localized) aleukemic lymphocytoma. Of the gastro-intestinal form of pseudoleukemia, Ewing makes the following observation:

"The gastro-intestinal tract is a seat of a remarkable form of primary lymphoid hyperplasia which lacks the destructive character of lymphosarcoma and fails to give lymphocytosis in the blood. The process may be chiefly limited to a portion or involve the whole of the gastro-intestinal tract or it may be associated with wide spread lesions of the most other lymphoid structures."

Wells and Maver[28] collected seven cases from the literature added one of their own and reported them under the title of pseudoleukemia gastro-intesinalis and thus, for the first time, brought to the attention of the pathologist and the clinician an interesting group of cases which they considered as "a division of the general group of cases that present the anatomical and symptom complex of Hodgkin's disease." Hayden and Appelbach,[11] however, considered these and other cases subsequently published under the same title, as belonging to an entity, entirely distinct from gastro-intestinal lymphogranulomatosis or Hodgkins disease.

Analyzed in the light of a more recent conception, at least a majority of these cases apparently represent a gastro-intestinal manifestation of lymphatic aleukemia and should be so designated rather than grouped under that ambiguous and misleading term, pseudoleukemia, which embraces also the other group represented by a minority of cases showing a sarcomatous transition or granulomatous nature. This assumption seems to be warranted from the histologic and hematologic data, meager as they are in most of the instances, afforded in the original reports cited by Wells and Maver and in those, subsequently, published by others. Thus, Carrington[3] reporting a case of Hodgkin's disease, stated "microscopically, sections of the stomach showed the structure of lymphoma, the growth consisting of abundant cells resembling leukocytes with, however, little or no evidence of reticulated stroma."

Hadden[10] described the growth as "strictly limited to the glands of the stomach without ulceration. On microscopic examination, the glands of the small intestine showed abundant small round cells, evidently a hyperplasia of the normal lymphatic tissue. There was no sign of invasion to neighboring parts." Pitt's[17] case showed microscopically, "the growths in the stomach

and elsewhere were found to consist of a dense collection of small cells in an adenoid reticulum which was with difficulty discoverable." The structure of the lesions in Symmer's[25] case was "peculiar, consisting of extensive multiplication of lymphoid follicles which became greatly enlarged and eventually fused—a true lymphadenoma, yet the cells in the centers of these aggregations of follicles were medium sized lymphocytes. Mitoses were missing. The process showed no capacity to invade resisting structures."

Stoerk[25] reported a case in which "by exclusion we come to a diagnosis of pseudoleukemia" and in which a microscopic study showed "the round cells forming the infiltration correspond in form and size and especially in size of the nucleus and protoplasmic content, to the type of lymphatic cells. In some places, there were found somewhat lighter cells which could not be differentiated from the cells of the adenoid tissue." On the other hand, he believed that there were some border line cases where he felt justified in making the following observation: "while in most of the cases, the cell types clearly resemble lymphatic cells, there are cases where the cells, because of their irregular forms, size of nuclei, non-uniform staining, resemble more sarcomatous than adenoid types. The problem is not easy to solve and we are probably justified to assume that we have to deal here with an intermediate stage between pseudoleukemia and lymphosarcoma, etc." In this class the case reported by Briggs and Elliott[2] might be more properly included while the case of Wells and Maver was an undoubted instance of Hodgkin's disease with chief manifestations in the gastro-intestinal tract.

From the foregoing discussion, it would seem reasonable to conclude that the case here reported is that of lymphatic aleukemia presenting major clinical symptoms referable to the stomach due to diffuse leukemic infiltration of the gastric wall without lymphemia or the involvement, at first, of the peripheral nodes. It exemplifies Ewings' true pseudoleukemia or systemic aleukemic lymphomatosis and Warthin's[14] generalized aleukemic lymphocytoma with its primary clinical and pathological manifestations in the stomach. Contrary to Warthin's experience that "lym-

phocytoma of the stomach or intestine and mesenteric glands has been one of the most common forms," search of references in the literature, supported by my experience and that of others, has shown that the condition comparable to my case, in all its essential features, seems strikingly rare.

Clinically, the majority of the patients suffering from this type of gastric lesion complain of vague symptoms referable to the stomach. Pyloric obstruction may be the chief presenting symptom. Free hydrochloric acid is absent in many of the cases reported. Correct diagnosis is probably impossible. With positive laboratory or roentgen findings, a presumptive diagnosis may be possible. Baldridge and Awe[1] observe that "when there is an actual invasion of the stomach wall by one of the non-circulating type [of lymphoma] the correct diagnosis may be established only by the discovery of an involvement of the peripheral lymph nodes .... without involvement of the peripheral lymph nodes the differentiation is apt to be impossible, clinically." Differential diagnosis from cancer, if at all possible, is of distinct therapeutic and prognostic importance. Differentiation between the various sub-groups of lymphoblastomata, if obtainable, may be of prognostic value. The roentgen rays offer the most direct method of visualizing the gastric lesion. Deformities and filling defects, thus visualized, are invariably interpreted as cancer. The case reported by Briggs and Elliott[2] may be cited as a typical example, presenting a characteristic roentgen picture of diffuse carcinoma of the stomach. Holmes, Dresser and Camp[12] reported a series of eight cases of "lymphoblastoma" involving the stomach. Of the six cases showing a definite filling defect in the wall of the stomach, five were interpreted as carcinoma and one as lymphoblastoma, the latter only in the light of a previous biopsy diagnosis on a peripheral lymph node. Demonstration of deep, heavy rugal impressions, by a proper technic, such as shown in our case of clinical lymphatic leukemia, cited above,[26] together with such pertinent clinical and laboratory data as a positive blood picture, biopsy diagnosis, etcetera, may be of diagnostic value. There is, however, no pathognomonic roentgen picture of the stomach in the gastric manifestation of this disease.

CONCLUSIONS

A case of lymphatic aleukemia showing an early involvement of the stomach with obstructive symptoms is reported.   The chief clinical interest lies in the fact that the condition resembled gastric cancer so closely that without necropsy, a correct diagnosis could not have been made.

Leukemic infiltration of the gastric wall with or without a positive blood picture, is a definite, though uncommon, clinical and pathological entity and should be differentiated from other forms of allied lymphomatous condition.

The term "pseudoleukemia gastro-intestinalis" represents, no longer, a distinct histopathological entity but merly a group of heterogeneous lymphomatous lesions of the digestive tract and should be discarded.

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EDITORIAL

Diphyllobothrium latum

The development and spread of civilization, knowledge, and commerce play decided parts in the infestation of both man and lower animals with animal parasites. A striking illustration of the transplantation of an animal parasite to a new country by lanes of travel is that of *Diphyllobothrium latum*, the broad tapeworm of man, which has been known for several centuries in Europe, in particular in the states bordering the Baltic sea, in Finland, northern Sweden, parts of Russia, Switzerland, and northern Germany. This parasite is also known in Japan. Evidence clearly points to its introduction into North America by immigrants, for in certain parts of Finland and Sweden every inhabitant harbors the worm. The first case in America, reported by Leidy in 1879, was that of a native of Sweden, resident in Pennsylvania but three months.

Although it has been known for many years that the larval stage of the worm lived as a small coiled white worm, in the muscle of certain fishes, in particular, pike, perch, pickerel and trout, it was not until 1917 that Janicki and Rosen demonstrated its complete life history. They showed that the egg passing in the feces from the adult worm hatched into a ciliated form known as a coracidium. This free-swimming animal, when eaten by certain copepoda, in Europe, *Cyclops strenuus*, grows in the body cavity into a small elongated worm after about two weeks. At this time the cyclops, if eaten by the proper fish, transmits the parasite by way of the fish's stomach into its musculature where the larva, known as a plerocercoid, awaits being eaten by a suitable mammalian host. It is obvious that only those who eat raw or undercooked fish could become infested with the parasite, and this explains the distribution of the worm, since it abounds in localities where it is the custom of the people to eat fish raw or

cold smoked. Perhaps the custom dates back to primitive fishermen, who even before the discovery of fire, ate their fresh, raw catches on the banks of lakes and streams.

Finlanders, Scandinavians and Russians, coming to this country, have settled in the middle western lake region and in Manitoba for there they find farming, fishing, trapping and iron mines, which furnish similar occupations to those pursued in their native lands. The ova of *Diphyllobothrium latum* found their way into the lakes at first accidentally; finally as the towns grew in size, they were deposited there through the agencies of modern sewerage systems, in large quantities. Essex, and Essex and Magath have demonstrated three copepods in this region capable of harboring the first larval stage, namely, *Diaptomus oregonensis*, *Diaptomus sicilis* and *Diaptomus siciloides* and Magath proved the presence of the plerocercoids in fishes from these lakes. Since then, these investigators, and Nicholson and Vergeer have shown pike, pickerel and perch from several lakes in northern Minnesota, from lakes in the northern peninsula of Michigan and from lakes in Manitoba and Alberta to be infested. Since most of the lakes involved belong to a chain which drains into Hudson's Bay it is possible some fish obtain their infestation in one lake and migrate to others. These areas correspond to the endemic centers of infestation in man previously established by Nickerson, Riley, Warthin and Magath. Sporadic cases have occurred in other parts of the country through the ingestion of raw fish obtained from these infested waters.

Although dogs take the infestation readily, their part in the spread of the worm may be questioned because ova obtained in their feces have very low vitality as compared with those in the feces of man, and their feces are often subjected to freezing and drying, both of which conditions kill the embryo in the ovum. Feces of dogs enter lakes for the most part accidentally.

The observation has been made that the greatest infestation in fishes occurs in lakes where sewage is emptied from towns in which Europeans who are accustomed to harbor the worm live, and the least infestation in lakes removed from habitation. This supports the contention that the parasite was brought to America

by Europeans and is maintained here largely through their continuing their custom of eating raw fish.

The fact that the larvae are killed in the flesh of fish when subjected to temperatures of $-9°C$. gives a sufficient cue to prevention of the spread of the parasite in man from a commercial standpoint. Any one may prevent his becoming infested by eating no raw or undercooked fish except it be fish from salt water. Diverting sewage from lakes and streams, unless it is rendered free from viable ova or larvae of *Diphyllobothrium latum*, will do much to prevent the spread and increase of infestation of fish. Fish should not be reared in hatcheries where the water is obtained from polluted streams or lakes.

Although a severe grade of anemia, almost exactly like primary anemia, has been described in persons who harbor *Diphyllobothrium latum*, the condition is very rare in America and occurs only in one person out of 5,000 to 10,000 in Finland, where the anemia is most often encountered. After a most careful evaluation of all factors, Birkeland, in a monograph which is in press, stated the conclusion that the worm acts as a "last straw to break the camel's back" in a person who is constitutionally susceptible to such blood dyscrasias.

The importance of this parasite in North America warrants a thorough educational program regarding it and an immediate attempt to control its development and spread.

<div align="right">T. B. M.</div>

SOCIETY NEWS AND NOTICES

Clinical pathologists will be interested in the following bills which were enacted during the first and second sessions of the Seventy-First Congress:

1. Public Law 361 increases the tariff rate from 45 per centum ad valorem to 55 per centum ad valorem on surgical instruments. This includes many instruments used by clinical pathologists such as hypodermic needles and syringes. Upon some syringes, as Record syringes, the rate has been increased to 70 per centum ad valorem. An increase of 20 per centum ad valorem has been made on many other scientific instruments which are chiefly composed of glass, whether for use in hospitals, laboratories, schools, or colleges. The rate on microscopes and such instruments has been increased 15 per centum ad valorem and on certain electrical devices and x-ray equipment the rate has been increased 5 per centum.

2. Public Law 106 provides for the coördination of public health activities of the Federal Government and Public Law 251 changes the name of the Hygienic Laboratory to the National Institute of Health. Public Law 251 further authorizes the acceptance of gifts by the National Institute of Health for the study, investigation and research in fundamental problems of the diseases of man. Individual scientists other than those connected with the Public Health Service may receive fellowships and may be appointed for duty to the National Institute of Health.

So far as can be learned there are no bills of particular interest to clinical pathologists being carried over to the present Congress.

In reviewing bills which were considered by state legislatures, three from New York State are of particular interest:

1. House Bill 2084, which was defeated, proposed to provide for the examination and licensing of laboratory technicians. A laboratory technician was defined as a person "who renders tech-

nical service and laboratory work designed and intended to be used as an aid in the investigation, prevention, diagnosis and/or treatment of diseases, or ailments of the human body, in a clinical research or public health laboratory or in a laboratory or office maintained by a physician or group of physicians in connection with his or their practice."

2. House Bill 157, which was defeated, was introduced to prohibit scientific experiments on living dogs.

3. House Bill 494, which was passed, provides for (1) the licensing by local health officers of persons, firms or corporations engaging in the business of procuring persons to donate human blood for transfusions, and (2) requiring that donors of blood present a certificate from a registered physician showing a satisfactory physical examination of the donor within ten days of the offering of the blood and his freedom from communicable disease.

The training of lay technicians was discussed by Dr. Walter E. King before the Congress of Medical Education, Medical Licensure, and Hospitals in Chicago in February, 1930. In his talk he summarized information concerning laboratories obtained in a questionnaire sent out by Dr. J. H. Black to medical schools and hospital laboratories. From this source of information it was learned that of eighty-one responding to the questionnaire fifty-four were engaged in training student technicians. There are sixteen colleges and medical schools giving regular courses for laboratory technicians varying from two months to four years, three of which lead to a degree. Dr. King pointed out the need for a better standardization of courses and urged the American Society of Clinical Pathologists to specify the minimum of work for students and the classification of technicians. The importance of didactic teaching as well as of laboratory practice was clearly brought out.

Full information concerning the next annual convention to be held June 8–9, in Philadelphia will be published in the May issue of the JOURNAL. Dr. R. A. Kilduffe, Chairman of the Program Committee, Atlantic City Hospital, is anxious that titles for

papers for the meeting be submitted to him at once, as the programs have become so long that it is necessary to make some selection of titles. No title will be accepted after May 15. Reservations should be made immediately with Hotel Adelphia, headquarters for the A. S. C. P.

At the 1930 convention of the Society it was decided that:

1. The Scientific Meeting and its discussions would not be reported stenographically as heretofore;

2. Discussions from the floor would not be routinely published with the papers;

3. That to be eligible for publication discussion must be given to the Secretary in writing.

These provisions are again called to the attention of the Society in order that those who desire their discussions recorded may properly prepare them for publication.

Those who intend to present papers, and who desire a careful discussion, are urged to furnish the Program Committee with a title and abstract *at the earliest possible moment* as only this can make arrangements for discussion possible. It is obvious that if only the title of the paper is given, or if title and abstract are not in the hands of the Committee until the last moment, any prepared discussion will be difficult to arrange. If any member presenting a paper desires to have a specific individual inaugurate the discussion, and will so advise the Program Committee, such arrangements will be made by the Committee.

Dr. W. S. Thomas, Chairman of the Committee on Exhibits, announces that two awards will be given this year. Reservations for exhibit space should be made early. Exhibits may be entered by non-members of the Society.

Attention is called to the letter sent out by the Research Committee on November 15, and to the postcard sent out by the Secretary on November 24, concerning the technic of the Huppert-Nakayama reaction. It is hoped that a general response to these communications will be made during the year.

Under the arrangements entered into with The Williams & Wilkins Company, part of the income from advertising and excess subscriptions is available for additional pages in the JOURNAL. It is, therefore, to the advantage of the Society to solicit subscriptions from others than members and to let the advertisers in the JOURNAL know that their advertisements are appreciated. Cooperation in this respect is urgently requested. In ordering materials from firms which advertise in the JOURNAL be sure to mention the fact that their advertisements were noted.

Members of the American Society of Clinical Pathologists are urgently requested to pay their annual dues immediately to Dr. A. S. Giordano, Secretary-Treasurer, South Bend, Indiana. This is particularly important at this time as the mailing list for the JOURNAL is made up of those members of the Society who are in good standing in regard to payment of dues.

Dr. I. Davidsohn has resigned the position of Director of Laboratories at Mt. Sinai Hospital in Philadelphia and has accepted the position of Director of Laboratories and Pathologist at the Mt. Sinai Hospital in Chicago.

Dr. Alvin G. Foord has resigned his position as pathologist at the Buffalo General Hospital to accept a position as pathologist for the Pasadena Hospital at Pasadena, California.

Dr. Edward F. Cooke of Houston, Texas died January 8, 1931, after a brief illness.

CORONARY OCCLUSION*

ERNEST SCOTT AND MARY K. HELZ

From the Laboratory of Pathology, Ohio State University

The terms "coronary occlusion" and "cardiac infarction" have, within very recent years, become familiar terms in medical literature and most of us are of the impression that this disease involving the coronary arteries and the cardiac muscle is a new condition that has arisen among us. If we will take time to investigate, however, we may find very complete descriptions of these changes in the literature of almost one hundred years ago.

Wearn[33] cites a case from the works of William Harvey published in 1845 in which a fairly accurate description of the clinical symptoms are presented. The record of the autopsy states that

"We found the wall of the left ventricle ruptured, having a vent in it of the size sufficient to almost admit any of my fingers although the wall itself appeared thick and strong; this laceration had apparently been caused by an impediment to the passage of the blood from the left ventricle into the arteries.

John Lindsay Stevans[27] in 1887 introduced his remarks by stating that

The conditions of the heart wall (especially fibrous transformation) have long been described by special observers, but it is only of recent years that what appears to be, in most cases, their true pathological significance has been hinted at.

This same author quotes Dr. W. T. Gairdner who, in 1854, reported a case of "ossification of the coronary arteries, with tendinous degeneration of the heart muscle." Many other references are cited by this author, who in his own discussion hinted at the acute conditions as "infarctions" and suggested that the coronary vessels played some part in the condition.

* Read before the Ninth Annual Convention of the American Society of Clinical Pathologists, Detroit, Michigan, June 20–23, 1930.

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In this country Dock[7] was the first to attempt the diagnosis of infarctions of the heart prior to its recognition at the postmortem, From this time on through the discussion of the subject by Osler,[24] Wearn, Levin,[21] Herrick[13] and many others, the relationship of the clinical manifestations and the accompanying pathology have become well understood.

The study of the pathology of cardiac infarctions leads to the necessity for a better understanding of the normal circulation of the coronary arteries, and in 1921 Louis Gross[11] published his monograph upon the "Blood Supply of the Heart," in which by new methods and better technique he was able to establish definitely the area of distribution of the coronary arteries and their main branches, the abundance of the capillary circulation, the free anastomosis between the capillaries and pre-capillary vessels, the exchange of blood not only between the branches of the same main artery but also between the right and the left coronary arteries. He further demonstrated the areas of over-lapping in the blood supply in different areas of the heart, the anastomosis of the vessels of the epicardial fat and other adjacent vessels with the coronaries, and that anastomosis between the coronary vessels increases with age.

In the distribution of the coronary vessels there are three areas in which the finer branches overlap, such areas receiving their supply from one or the other artery depending upon the pressure at any given time. Kugel[17] has demonstrated the constant presence in fifty human hearts of the large anastomotic artery, first described by Kugel and Gross, passing through the auricular wall and the interauricular septum which joins the left and right coronary arteries. He has named this the arteria anastomotica auricularis magna. Oberhelman and LeCount,[23] by injection methods followed by roentgenograms, were able to demonstrate that in a few hearts no anastomosis exists. In a second group of normal hearts there was a rapid and complete filling of both coronary arteries following the injection of either one. In a third group, in which there were extensive myocardial changes and an advanced coronary sclerosis, there was shown an abundant anastomosis. These authors suggest two factors concerned with the

results of coronary sclerosis; first, the considerable difference in the anastomosis normally existing between the coronary vessels, and second, the time element (whether occlusion has taken place slowly enough that collateral circulation may be established). In a series of experiments conducted in this laboratory it was found that, following the injection of normal saline into either coronary vessel at a pressure of 100 mm. mercury, 1–2 per cent of the fluid was collected from the canula in the opposite coronary orifice.

Gross and Wearn[32] by means of injecting colored fluid into the coronary vessels, have been able to demonstrate an unusually abundant capillary circulation in the normal heart muscle. Wearn[31] was able to prove a capillary for practically every fiber of the ventricle walls and the papillary muscle, though a less abundant supply in the auricle and Purkinje system. This abundance of the capillary circulation can in some measure be realized when the cast of the coronary vessel is obtained by the celloidin injection and corrosion method. Here an extremely fine meshwork of capillaries can be seen so closely packed together that there seems that no space is left for the muscle fibers. Such a cast of the heart vessels also demonstrates the course and distribution of the branches showing the acute angle at which the branches of the left coronary artery leaves the vessel to pierce the muscle.

The Thebesian vessels since their very early description by Vieussens[20] in 1706 and by Thebesius[29] two years later, have created great interest and much difference of opinion as to their function and distribution. Within more recent years Pratt,[25] Crainicanu,[6] Wearn,[31] Grant and Viko[10] have continued these investigations. Pratt first succeeded in demonstrating that it was possible to maintain the beating of the heart by perfusing blood through the Thebesian vessels. Crainicanu observed that salt solution perfused through the coronary arteries after the canulation of the various openings of the heart, the aorta, the pulmonary vein, coronary veins, coronary sinus, and the auricles, that most of the solution escaped into the chambers of the heart and that only a small amount escaped through the coronary sinus. Wearn

in extending these observations, noted that as much as 90 per cent of the fluid introduced into the coronary orifices escaped through the cavities via the Thebesian vessels. He was also able in this manner and by serial sections of the heart to demonstrate connections between capillaries and the Thebesian vessels, and a direct connection between the coronary arteries and the chambers of the heart via the Thebesian vessels.

Grant and Viko, by injections of chrom yellow gelatin and celloidin of varying viscosities into the coronary arteries and also into the Thebesian vessels through their opening in the endocardium, and later by either clearing or digesting the muscle, describe three main types of vessels arising from Thebesian foramina:

1. Vessels forming "trees" and ramifying in the endocardium and the immediately underlying vessels.
2. Channels uniting neighboring foramina and anastomosis between adjacent "trees."
3 Vessels connecting the Thebesian "true" with the coronary veins, and also direct communication of coronary vein and the ventricle.

They conclude their report with the positive statement that the Thebesian vessels communicate with the coronary arteries only through the capillaries.

This brief review of the coronary circulation indicates that the question of the heart's nourishment has occupied the attention of medical investigators from the dawn of anatomical study, and that while much has been learned, the entire mechanism through which the myocardium is fed is still in doubt. This has recently been brought to attention by the report of Leary and Wearn[8] who report two cases of complete closure of the orifices of both the left and right coronary arteries by the thickening of the aortic wall from a syphilitic aortitis, and in our own laboratory there is at present a similar case in which both coronary orifices are so completely closed that no fluid can be forced through them.

Again after such a review of the anatomical peculiarities of the heart's circulation, the question unavoidably asked is why should there be infarcts at all in an organ with such free and abundant

anastomosis between its arteries and in which even the complete closure of one or both branches of its usual sources of supply has been shown without seriously impairing the function of the organ. As an explanation for the question Pratt has postulated the presence of "functional endarteries" or areas in which the resistance is greater than the surrounding pressure. Hirsch and Spalteholz[15] have shown experimentally that the area of infarction is always smaller than the area supplied by the affected vessel, when however, the blood pressure was lowered or poorer in quality the infarct included the entire area of the vessel's supply. He believes that the continuous functioning of the heart, the structure of the vessels and the heart strength all bear a definite relationship to cardiac infarction. In this consideration it must be again noted that the age of the individual, the size of the vessel affected, and the rapidity with which occlusion occurs are matters of utmost importance. For if obliteration is gradual and the circulation good, anastomosis and collateral circulation may be sufficient to maintain the life of the areas involved. Such an abundant collateral circulation may be established that the myocardium maintains its normal appearance and function even when arteries of fair size are closed. Such cases are recorded by Gross and Galli.[8] If, on the other hand, the circulation is partially cut off, it may still be sufficient to maintain the life of the fibrous tissue or stroma of the part, while the more delicate muscle cells will degenerate or disappear through atrophy.

With sudden closure of a large artery there is not time for the establishment of any adequate collateral circulation and the entire area quickly becomes necrotic (myomylaciae cordis).

The deciding factors in the amount and kind of infarction in the heart muscle are the size of the vessel, the rapidity with which occlusion occurs, the condition of the general circulation, and the age of the individual.

The most common age at which coronary disease manifests itself clinically corresponds with that at which it is observed at the autopsy. Herrick and Nuzum[14] note that 82 per cent of cases dying of coronary disease fall in the age range of 40–70 years, while 44 per cent come between 50–60 years. Barker[4] finds the

average age about 54 years with the individual usually over 45. Levine[20] finds the disease mostly in men 55 or over. The average age of Allen's[2] cases is 55.4 years. Cases accompanied by a syphilitic process fall in the earlier age limits. These seem to be 35–55 years on the average. All other cases fall in the upper range averaging around 50–60 years.

All writers agreed that men are more often affected than women, but the earlier idea expressed by Osler[24] that the upper social stratum is predisposed, is being corrected as more cases are studied. Wearn and Levine both cite many cases from the lower levels of society, and in our own series of 36 cases there were two ministers, one physician, one business man, one woman of high social standing; the remaining 31 are classed as common laborers and housewives from the poorer class.

The relation of coronary disease to syphilis is a much debated question. According to Levine[19] syphilis is apparently not an etiological factor in acute cardiac upsets. In Thayer's[28] twenty-four cases of angina pectoris, four showed luetic aortitis only, while five showed luetic aortitis and coronary disease. Levine cites five cases of coronary occlusion, one of which was luetic. Allen collected 371 cases of coronary disease from 1000 consecutive autopsies. Of these, thirteen cases (3.5 per cent), showed some evidence of syphilis of the coronaries *"at or near the orifice."* Seven of his ninety-seven cases which showed narrowing or blocking of the coronary lumen, belonged in this luetic class, and eleven of the fifty-eight cases of coronary occlusion with sudden death showed this type of syphilitic process.

From the author's description of these cases, it seems that the syphilitic process in the aorta, either extends for a short distance into the orifice of the artery, or the proliferation in the wall of the aorta itself in the region of the sinus of Valsalva mechanically occludes the coronary orifice. These two modes of occlusion are apparently the only ones described in literature directly attributed to syphilis, there being no reference to a true syphilitic degeneration of the walls of the coronary vessels.

In considering coronary disease and angina pectoris care must be taken to distinguish between angina-like pain and true an-

gina.[1] Acute coronary obstruction is often accompanied by severe pain similar to angina. However, there are a goodly number of cases on record in which the typical attacks of angina are described. Some suffer repeated attacks in one of which they die, and at autopsy an acute coronary obstruction can be demonstrated. In other cases there is but one typical anginal attack during which the patient dies. Autopsy in some of these cases also reveals an acute cardiac infarction. Herrick mentions several such cases. Mackenzie,[22] in his work on angina, notices often a diminished blood supply to the myocardium, this being caused in a few cases by aortitis, but most often by coronary disease. He suggests that the pain might be due to a too great burden placed upon the weakened myocardium. Gilbert[9] sees coronary disease often associated with angina pectoris but considers it by no means a causative factor. Albutt[3] on the other hand, believes coronary occlusion has nothing to do with angina pectoris.

In the literature reviewed no direct mention is made of the relation of coronary disease to hypertension, though Levine, Hamburger[12] and many others note enlarged hearts in these patients. Wearn could not establish a direct relationship between the two conditions though they were often found associated. It is therefore interesting to note that in five of the present series hypertension was specifically diagnosed. The lack of symptoms and the suddenness of many deaths in coronary occlusion probably account for the scarcity of data on these attendant conditions, many of these patients never consulting a physician until in the throes of the terminal attack.

Again general arteriosclerosis is an attendant condition which has been seldom mentioned in the discussions of coronary disease, yet its relationship to this condition was indicated by Stevens as early as 1887. The frequency with which it occurred in the present series (44 per cent) would indicate that this condition should be given greater consideration in the etiology of the disease.

In Allen's 371 cases of coronary disease, sudden death from coronary occlusion occurred in only 58 cases. Such occlusion may

take place by several different methods, the most common being that of thrombosis. Any pathological process which tends to roughen the inner surface of a vessel predisposes to thrombus formation. According to Allen atheroma is the commonest of such lesions. An associated calcification complicates the picture in many instances. An arteriosclerotic process with or without calcification also predisposes to thrombus formation. These degenerative processes are quite often diffuse throughout the coronary system, but as Wearn, Allen, and others have pointed out, and as is indicated in the present series, the actual thrombosis occurs in the great majority of cases in the descending branch of the left coronary artery. Syphilitic lesions are proliferative in character and, occurring at the base of the aorta, tend to obliterate the vessel orifice. This type of closure is so gradual that an acute infarction rarely occurs. Albutt cites several such instances of almost complete occlusion of the coronaries. Leary and Wearn's two cases of complete occlusion of both coronary orifices by a syphilitic aortitis and our own similar case have already been referred to.

Occlusion may also be due to an embolus although this does not presuppose coronary disease. The embolus may be paradoxial or may originate in the left heart itself. In one of the cases in the present series the embolus arose from a thrombus in the right femoral vein and reached the left coronary by way of a patent foramen ovale. In another, there was an embolus in the left coronary and a patent foramen ovale, but the origin of the embolus could not be determined. In a third case the embolus arose from a clot in an aneurysm of the ascending aorta.

In order that there may be a definite understanding of the microscopic changes in coronary disease, we have adopted the classification followed by Allen which coincides with our views on the subject. His division is that of (1) atheroma, (2) 'arteriosclerosis, (3) calcification, and (4) syphilis.

The atheroma originates as an inflammatory process in the deep layers of the intima, and later, becoming fibrous, it produces nodular encroachments upon the lumen. This disease in the coronaries is quite comparable to atheroma of the aorta. It is rather prone to calcereous degeneration in its later stages.

Arteriosclerosis is a diffuse process probably originating in the medial and sub-intimal layers. It leads to a diffuse fibrosis with thickening of the media, sometimes extending into the intima. This is also prone to calcereous degeneration.

While syphilitic aortitis is a common manifestation of syphilis, the disease seems rarely to attack the coronaries. A syphilitic mesaortitis may encroach on the lumen of the coronaries at their orifices or as has been stated before, may extend a short distance into the coronary. However, in the present series, there is one case presenting proliferation and fibrosis of the intima accompanied by a round cell infiltration which seems to originate in the adventitia and extend into the media. The appearance is quite similar to the accepted picture of syphilitic aortitis and syphilis of the medium sized vessels as described by Saphir.[25]

The alterations in circulation due to coronary obstruction can be divided into two main classifications, namely those following sudden obstruction and those due to gradual closure. An acute infarction follows sudden obstruction. Oberhelman and Le-Count found that in a few hearts there was no vascular anastomosis. As they also pointed out, a sudden occlusion, especially of a fairly large branch, does not give time for functional anastomosis and myocardial ischaemia is the result. In the event of this too sudden interference, death ensues. Gross points out that functional anastomosis may carry enough blood to proliferate and nourish fibrous tissue and thus conserve the life of the patient, while it is not sufficient to the needs of highly specialized muscle.

In sudden occlusion the microscopic picture in the myocardium is one of acute infarction. Karsner and Dwyer[16] in their experiments upon dogs found that by surgically ligating a coronary artery, infarctions could be produced. These dogs were allowed to live for varying lengths of time and then the heart removed for examination. By knowing the exact age of the infarct, the progressive myocardial changes could be studies. Thus they have a complete myocardial picture from the occlusion until complete healing by fibrosis. Buckley[3] finds cardiac infarction responsible for most cases of spontaneous rupture of the heart.

However, this is not the most common end result of infarction. A great many infarctions end in fibrosis. This is especially true when smaller areas are affected.

The most common type of vascular obstruction in the heart is that which takes place gradually. Here the collateral circulation plays a great part because we see no acute infarction. The new circulation, unable to carry enough blood for all the myocardium, can support the growth of connective tissue. Mackenzie, Wearn, Hamburger, and Allen all agree that fibrosis is the usual result of gradual closure of the coronary arteries. Mackenzie goes on to say that if this fibrosis is extensive enough, death may follow from progressive myocardial failure, the heart muscle being unable to compensate for the extra strain placed upon it. In our experience the most common picture is an acute infarction superimposed upon a chronic fibrosis. The coronary picture is one of a narrowed lumen suddenly blocked by a thrombus. A syphilitic process which gradually obstructs the coronary orifice seems to lead to fibrosis less often, according to Allen.

ANALYSIS OF THIRTY-SIX CASES OF CORONARY OCCLUSION

Age:
  Average age................................... 57.5 years
  Average age of syphilitics........................ 51.6 years
Sex:
  Males... 32
  Females.. 4
Race:
  White... 31
  Black.. 5
Associated clinical findings:
  Angina pectoris diagnosed........................... 4
  Hypertension diagnosed.............................. 5
  Hypertrophy and dilatation of heart................. 3
  Cerebral hemorrhage................................. 3
  Sudden death....................................... 22
  Death in 10 to 12 hours............................. 2
  Slow death.. 7
  Manner of death unknown............................. 5
  Pain... 11

Myocardium:
    Acute infarction superimposed upon fibrosis.............. 16
    Fibrosis....................................... 5
    Acute infarction............................... 8
    Acute infarction with rupture.................... 2
Vascular disease:
    General sclerosis.............................. 17
    Syphilitic aorta................................ 16
    Atheromatous aorta............................ 3
    Arteriosclerotic aorta.......................... 11
    No aortic disease noted........................ 6
    Coronary arteries:
        Occlusion main branch left coronary.............. 11
        Occlusion bifurcation left coronary.................. 2
        Occlusion descending branch left coronary........... 15
        Occlusion intermuscular branches left descending
            coronary...................................... 2
        Occlusion right coronary....................... 5
        Occlusion both coronaries........................ 1
    Manner of final occlusion:
        Thrombus..................................... 29
        Embolus...................................... 3
        Syphilis....................................... 1
        Manner of occlusion unknown..................... 3
    Coronary disease:
        Arteriosclerosis................................ 16
        Atheroma..................................... 1
        Syphilis....................................... 3
        Arteriosclerosis and calcification.................. 7
        Atheroma and calcification...................... 5
General syphilis:
    A diagnosis of syphilis was possible in.................. 17
    A doubtful diagnosis in............................ 1

SUMMARY

1. In distinction from other series of cases the present series showed seventeen (47.2 per cent) syphilitics.

2. Virtually all cases in the series were accompanied by a general vascular disease.

3. In thirty of the thirty six cases the left coronary was occluded.

4. The right coronary was occluded in only five cases.

5. In one case both coronaries were completely occluded.

6. The most common coronary lesion was arteriosclerosis with superimposed thrombosis.

7. The most common myocardial lesion was an acute infarction superimposed upon chronic fibrosis.

8. The most frequent victim of coronary occlusion was a white male 50–60 years of age.

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THE ELECTROMOTIVE THERMOMETER

An Instrument and a Method for Measuring Intramural, Intravenous, Superficial and Cavity Temperatures*

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Seebeck, of Berlin, in 1821, discovered that if, in a circuit made of two different metals, one junction is hotter than the other, an electromotive force is developed which causes an electric current. This electromotive force is generally very small compared with ordinary single unit batteries and the like, which have voltages of from 1 volt to 2 volts. For example, in a circuit containing copper and iron (hence two junctions), when one junction is at 100°C. and the other junction is at 0°C. the electromotive force is of the order of a thousandth of a volt. This voltage, however, causes an electric current to flow from copper to iron at the hotter junction and from iron to copper at the colder one when the electric circuit is closed.

In order to obtain larger electromotive forces, pairs of metals have been combined in series to form thermopiles. The form devised by Nobili and used by Melloni in his researches on heat radiation consisted of alternate strips of antimony and bismuth which were insulated carefully from each other, except at the junctions, where they were soldered together. These metals were chosen because they gave a large electromotive force which acted from bismuth to antimony at the hot junction and from antimony to bismuth at the cold junction.

I do not know to whom is due the credit for the initial use of thermocouples and thermopiles in obtaining measurements on

the temperatures in various parts of the human body. The first reference I have been able to find is a paper by Lombard.[11] Lombard employed thermopiles of eight pairs of bismuth and of an alloy of antimony and zinc. In the same year and journal, Allbutt[2] published a short paper on the clinical thermopile. He used thermopiles of bismuth and antimony. He gave some reports of his clinical observations and wrote by way of conclusion: "Perhaps the most valuable results I have obtained are the relations between superficial and internal heat."

Passing over the rather sparse medical literature dealing with thermocouples and thermopiles for the determination of the temperatures of body surfaces and tissues from 1875 to 1915, I shall call attention briefly to some of the more recent investigations.

Holding[10] described his thermocouple thermometer as an instrument "for the exact measurement of temperature in living tissues for use in coagulation surgical procedures." He employed a potentiometric method for reading the electromotive forces developed by the heat of the tissues, particularly in procedures involving diathermy and electrocoagulation.

Crile[7,8,9] and his collaborators made a series of thermo-electric studies of variations in temperature in animal tissues, the effects of anesthesia, electrical stimulation, abdominal trauma, exposure of viscera, excision of organs, acid, alkali, strychnine and epinephrine.

Wagner[13] described the construction and use of thermoneedles for measuring the temperature of deep lying organs and used such thermocouples in tumors, muscles and the like.

Bazett and McGlone[4] employed couples of constantan and iron for the measurement of superficial temperatures and used steel needles with constantan for dermal and subcutaneous measurements. One of the junctions in their portable apparatus was inserted in an insulated (thermos) flask of a capacity of one pint and filled with paraffin oil at room temperature. They used a galvanometer of the double pivoted movable coil type (Weston, Model 440), of internal resistance of 3.5 ohms, period of 2.3 seconds and deflections indicating 2.2 microamperes in each division. The sensitivity of their ensemble was between 0.6 and

0.8°C. for each division of the scale, insuring an accuracy of about 0.2°C. in the estimation of temperature by their thermo-electric method.

In various topographical surveys of the temperatures of the body at numerous (forty-seven) cutaneous situations, Benedict,[4] and Benedict, Koropatchinsky and Finn[5] used thermocouples of constantan and iron, a thermostat for maintaining one junction at a constant temperature (32°C.) and a portable galvanometer (Leeds and Northrup number 2400), with a sensitivity of 20 microvolts, which indicated a change of temperature of practically 0.04°C. for each millimeter of deflection of the galvanometer. There are many valuable details concerning construction of apparatus and methods of obtaining temperatures in these papers by Benedict and his collaborators.

Clark[6] designed and built a sensitive apparatus (reading to 0.01° C.) for the measurement of intravenous temperatures employing thermocouples of constantan and copper. He made a thermostat consisting of a double flask or tubes of pyrex glass, the space between being filled with mercury which served both as the constant temperature bath and, by the change of volume of the mercury and subsequent change of level in the capillary tubes attached to the apparatus, as the regulator of the temperature. The thermostatic apparatus which I have used in the electromotive thermometer is a modification of the device of Clark.

THE FUNDAMENTAL PRINCIPLE OF THE ELECTROMOTIVE THERMOMETER

The electromotive thermometer is based on the physical principle that an electromotive force (voltage) is developed in a circuit which consists of junctions of two different metals, such as constantan and copper, when one junction or thermocouple is at a higher temperature than the other junction. If a galvanometer is included in this circuit, the electromotive force developed by reason of the differences in temperature of the two junctions will cause an electrical current to flow through the galvanometer. If, then, the difference between the temperatures of the two junctions is known, it is possible to calibrate or to convert the galvano-

metric deflections into equivalent thermal readings. Having calibrated the deflections of the galvanometer in equivalent thermal readings, any of the various thermocouples with which the apparatus is equipped may be applied to or inserted into the body and the temperature read from the calibrated scale, provided the value of the resistance in each of the thermocouple circuits is the same. In passing, it should be noted that the thermocouple, whether bare or inserted in a needle or other enclosing capsule (as in the rectal or gastric thermocouple) should remove as little heat as possible from the surrounding tissue if it is to indicate accurately the temperature of the tissue. In many instances it is difficult to accomplish this: therefore, thermocouples in some cases will be more significant as indicators of the change of temperature produced or induced by various clinical or surgical procedures.

THE ELECTROMOTIVE THERMOMETER

The ensemble of apparatus consists essentially of two parts: (1) the thermostat and heating circuit so arranged that one set of junctions (constantan, copper) or, in the lastest type of instrument, one common junction, can be kept at a constant temperature, and (2) the various types of thermocouples for use in the determination of intramural, intravenous, superficial and cavity temperatures. The galvanometer, used as the instrument for the measurement of the difference in electromotive forces of the two junctions (as for example, the junction at a constant temperature in the thermostat and the junction applied to a given area on the skin) may be of any degree of sensitivity desired, dependent on the distance of the scale on which the deflections are read and the degree of accuracy of measurements desired.

The thermostat

This is essentially a modification of the double container or flask of pyrex glass designed by Clark. A photograph of the thermostatic flask which is used in the apparatus is shown in figure 1. It is shown also in cross section as a portion of figure 2. The length of the double flask portion is 7 inches; the
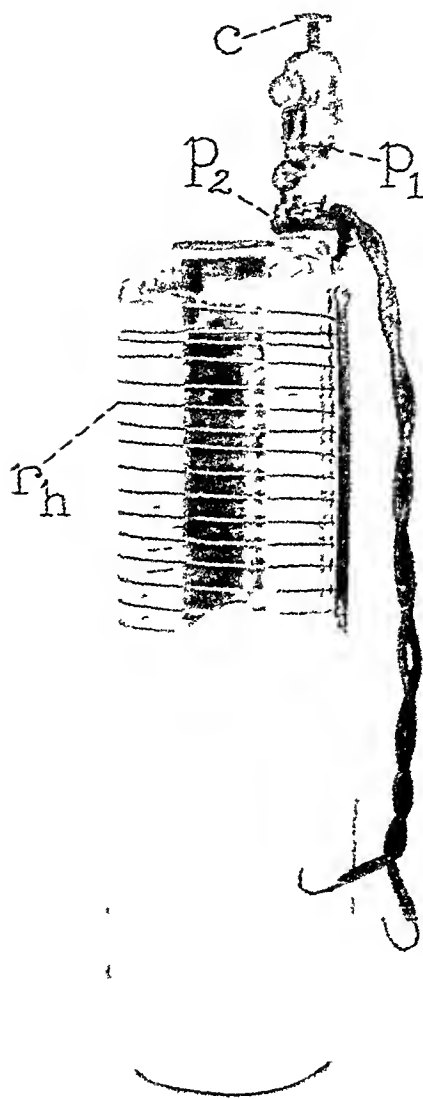
FIG. 1. THE PYREX DOUBLE FLASK OR THERMOSTAT

p_1 and p_2, platinum wires which, with mercury, form the thermostatic control: c, the adjustable screw to which is attached one platinum wire and which serves as the regulator of the value of the constant temperature of the thermostat: r_h, the resistance which constitutes the electrical heating system of the thermostat.

diameter of the outer tube is 2 inches. In the center of this outer flask there is sealed a second tube, about 1 inch in diameter. At

the top of this double flask, and toward the outer edge, there is sealed a piece of pyrex capillary tubing of 1.5 to 2 mm. internal bore and 1 inch in length. The upper end of this capillary tube is flared or cupped. Over the top of this cup is fitted a metallic cap and screw (fig. 2, c). At the end of the screw is a small platinum wire (fig. 2, p) which makes contact with the mercury

Electromotive Thermometer

Thermostat and heating circuit | Thermocouple and galvanometer circuit
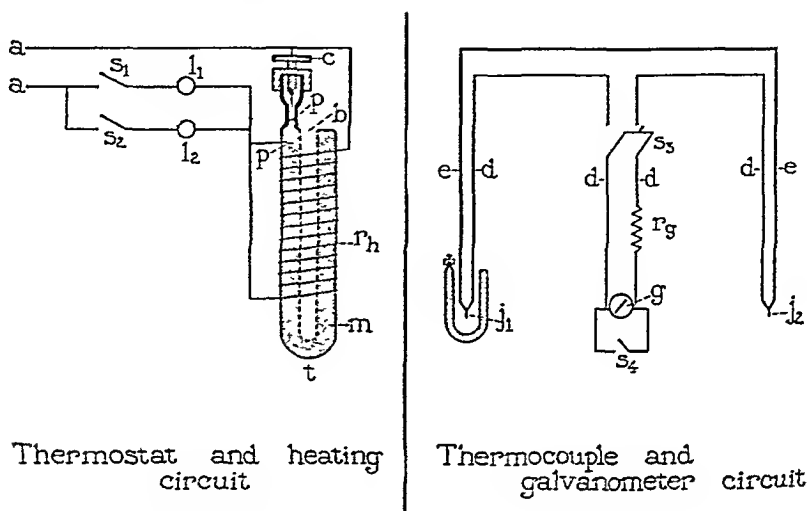
FIG. 2. DIAGRAMMATIC SKETCHES OF THE ELECTROMOTIVE THERMOMETER

Left-hand diagram: a–a, 110 volt circuit; s_1 and s_2, switches for inserting auxiliary resistances (incandescent lamp bulbs) l_1 and l_2 in the circuit; c, adjustable screw; p–p, platinum wires which, with mercury, form the thermostatic control; b, inner tube of thermostat which carries one set of thermojunctions; m, mercury; r_h, resistance for heating thermostat.

Right-hand diagram: j_1, j_2, thermojunctions of copper, d, and constantan, c; r_o, auxiliary resistance in galvanometric system; g, galvanometer; s_3, switch for including the galvanometer in the thermo-electric circuit; s_4, shunt to damp the galvanometer quickly.

(fig. 2, m) as it expands under the heat derived from the electrical heating of the thermostat (fig. 2, r_h). By means of the adjustment of the screw (fig. 2, c) the temperature of the thermostat may be set at any value desired. The thermostat proper (fig. 2, t) is wound with about 15 feet of number 24 "advance" wire (Driver-Harris Co., Newark, New Jersey) and has a resistance

of about 10 ohms. It is then coated with plaster of paris to a depth of about $\frac{1}{4}$ inch and is finally inserted with proper insulation in the metallic case which is furnished with thermos bottles. The glass portions of the thermostat may be obtained from the Central Scientific Company, Chicago.

The heating circuit

The thermostat is electrically connected to the usual 110 volt lighting circuit. Two ordinary incandescent lamps (or more if desired) are arranged in parallel. One or both of these lamps may be included in the circuit by means of switches (fig. 2, s_1 and s_2). Initially, both switches are closed so that the temperature of the mercury in the thermostat and hence the temperature of the oil in the inner tube (fig. 2, b) may be raised to the desired value as quickly as possible. If the screw (fig. 2, c) has been set by previous trial to cause the heating circuit to be "off" at a temperature of 40°C., it is possible to insert a Centigrade thermometer accurately calibrated in tenths of a degree (range to 50°C.) in the inner flask (fig. 2, b) and, when the temperature indicated on this thermometer rises to within a few degrees of 40°C., the switch (fig. 2, s_2) can be opened and the temperature of the thermostat allowed to come more slowly to its fixed or constant value. The temperature of the thermostat can be kept constant to within less than 0.05°C. if redistilled mercury is used in filling the double-flask container. As will be seen by reference to figure 2 (left-hand portion), the electric current from the main supply passes through the resistance r_h until such time as the mercury has been heated sufficiently and has expanded to make contact with the platinum wire (p in upper right-hand portion of fig. 2) attached to the adjusting screw (fig. 2, c). When this contact occurs, the electrical current does not pass through the resistance r_h, but follows the path a–c–p–p–l_1–s_1–a (fig. 2). If arcing occurs at the break of the contact of the platinum point of the adjusting screw (fig. 2, c), a satisfactory remedy will be found in the employment of condensers (such as telephone condensers) placed across the mercury-platinum "make and break" points.

The thermostat and heating circuit

Figure 3 is a photograph of the arrangement of lamps (fig. 3, l_1 and l_2) and the situation of the thermostatic control in the electromotive thermometer. The current, after passing through the lamp or lamps in the circuit, goes through the resistance wire
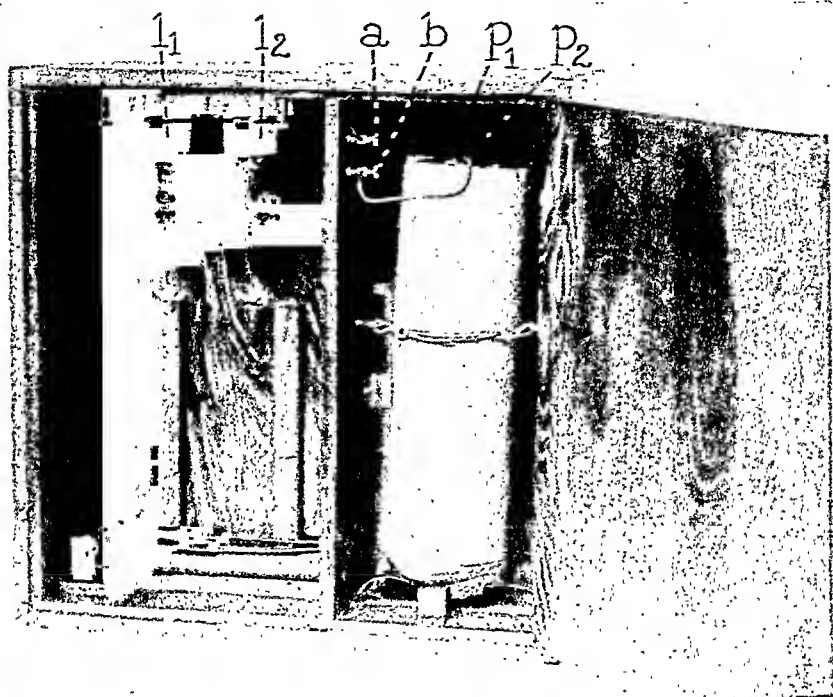
FIG. 3. PHOTOGRAPH OF THERMOSTAT AND ELECTRICAL CIRCUIT

l_1 and l_2, lamps; p_1 and p_2, adjustable screw and platinum wires of thermostat; a and b, leads from p_1 and p_2 to main 110 volt circuit.

wound around the pyrex double flask containing the mercury. The points marked a and b (fig. 3) indicate the binding posts to which are fastened wires leading to the two platinum contacts (fig. 3, p_1 and p_2).

The thermocouples

These are made of copper and constantan wires. The copper wire used is well insulated, B and S gauge 27, and consists of

seven number 35 wires, tinned and laid straight. It is commercially sold under the code name "Habitual" (Belden Manufacturing Co., Chicago). The constantan wire is made by the Driver-Harris Company, Newark, New Jersey, and is sold under various trade names, such as "Ideal" and "Climax." A flexible, well insulated wire made of four strands number 35 or 36 is
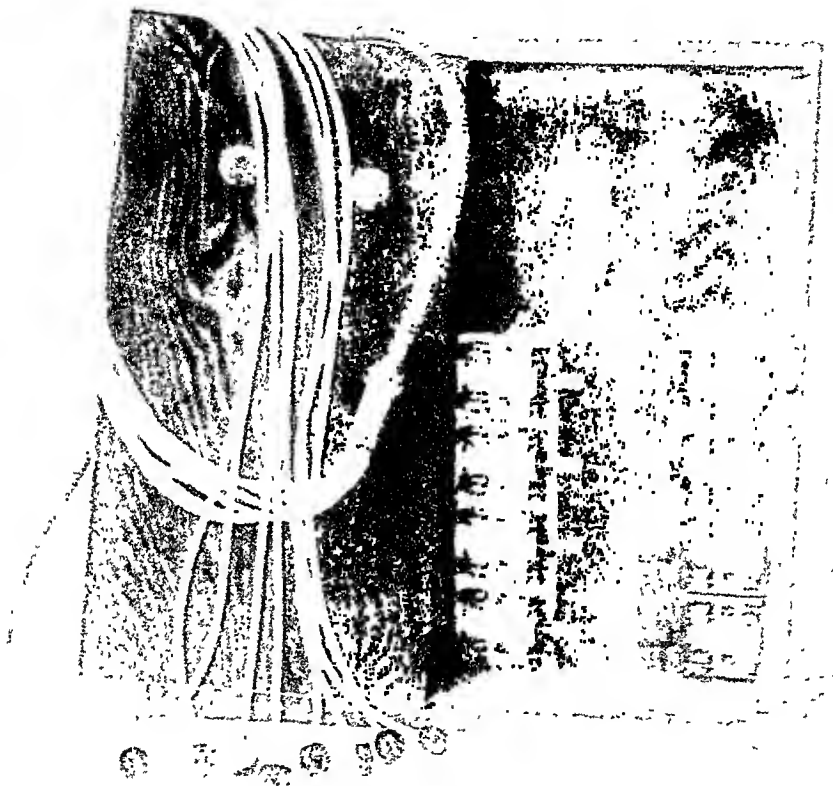
FIG. 4. PHOTOGRAPH OF SKIN THERMOCOUPLES AND SWITCHES CONNECTED TO EACH PAIR OF THERMOCOUPLES RESPECTIVELY

suitable. It is an advantage to use stranded wire of such an alloy, since it is less brittle and less likely to break than the non-stranded type. Also, by using wires of these diameters it is possible thoroughly to interweave and wrap the ends of the copper and constantan wires together for about a quarter of an inch before applying a drop of solder at the tip.

In the earlier models (constructed from 1925 to 1929) one of

each of the various pairs of copper-constantan thermocouples was inserted in the thermostat, and the other junction and wires, forming the second of each pair of thermocouples, were led through several (generally six) feet of rubber tubing of about the same size as the smallest Rehfus tubing (fig. 4). Each pair of thermo- couples was connected to the galvanometer in the manner indi- cated in the right-hand diagram of figure 2. In certain types of experiments (with diathermy), however, some difficulties arose by reason of the fact that the insertion of ten or more thermocouples
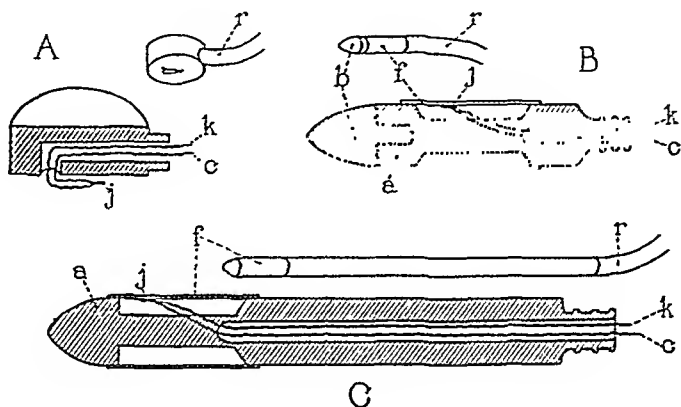
FIG. 5. DIAGRAMS TO SHOW CONSTRUCTION OF CUTANEOUS THERMOCOUPLE A, GASTRIC THERMOCOUPLE, B, AND RECTAL THERMOCOUPLE, C.

k, constantan; c, copper; a, fiber or hard rubber; b, metal cap; f, thin copper tubing; r, rubber tubing; j, thermojunction.

in the inner flask of the thermostat caused these thermocouples to come in contact with each other. This contact was prevented by inserting each thermojunction (in the thermostat) into a small glass tube. The arrangement of switches for enabling the operator to complete the circuit of any desired pair of thermojunctions through the galvanometer, and thereby to get a reading of the temperature indicated by any given thermocouple is shown in figure 4. A group of thermocouples suitable for use in the measure- ment of cutaneous temperatures is also shown in the same photo- graph.

Figures 5 and 6 are a series of sketches indicating the manner of construction and housing of thermocouples to be used in ob-

taining superficial, intravenous, intramural, or cavity temperatures. In the measurement of gastric or rectal temperatures it is essential that the thermocouple come nearly in contact with a very thin, small, metallic shell which serves as the container so that it may register as quickly and accurately as possible the temperature of the tissue with which it is in contact. Thermocouples can be made interchangeable also, as for example those used in intravenous, rectal, and gastric measurements, if the copper and constantan leads are fastened respectively to metallic
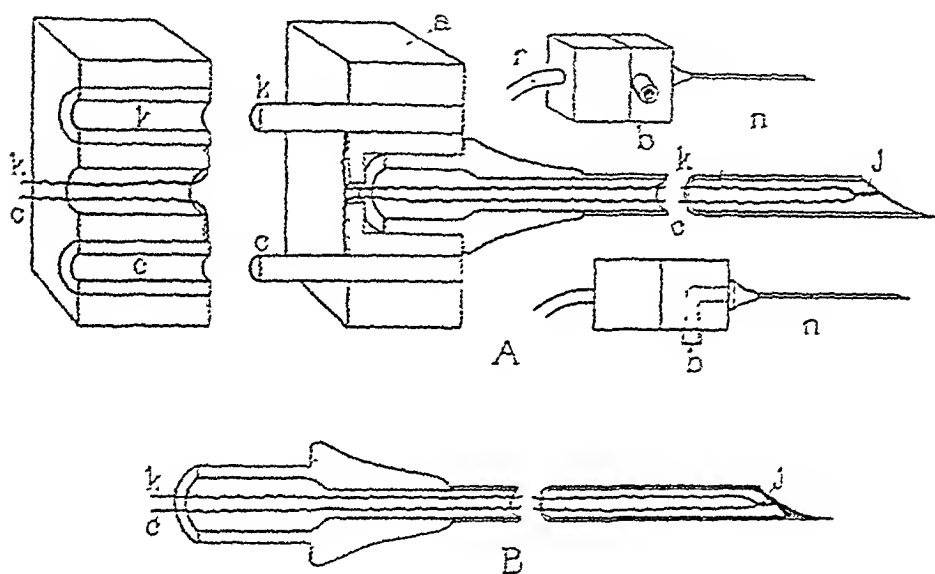
FIG. 6. DIAGRAMS TO SHOW CONSTRUCTION OF INTRAVENOUS THERMOCOUPLE, A, AND INTRAMUSCULAR OR INTRAMURAL THERMOCOUPLE, B

k, constantan; c, copper; r, rubber tubing; b, attachment for bulb; n. needle, and j, thermojunction.

bushings of copper and constantan. The attachment containing the thermocouple proper can be conveniently joined by a jack, the constantan lead always being inserted in the bushing of constantan. The essential point to be emphasized is that matters shall be so arranged that two junctions of dissimilar metals occur at two points only; namely, the copper-constantan junction in the thermostat, and the similar junction in the needle or other holder to be inserted into or attached to the body.

The galvanometer

Any galvanometer of the d'Arsonval type, and of sufficient sensitivity, may be used. A Leeds and Northrup type R galvanometer 2500-a has been found satisfactory. If the galvanometer is too sensitive, a resistance of suitable value (fig. 2, *r*) may be inserted in series with it. The external circuit from the thermostat through the switches to the galvanometer, including binding posts, wire terminals, resistances, and so forth, are of copper. All metallic parts in the circuit (fig. 2, *d–d*), or similar circuits, are of copper. In general, I have mounted the galvanometer on one wall of a room in which the instrument is to be used and have used a lamp house with an image of a wire attached to the housing (or the image of the filament of a single filament lamp may be employed) as an indicator of the galvanometric deflection. The scale, calibrated in degrees and fractions thereof, may be fastened to a wall or other support at some distance from the galvanometer. Such a scheme makes the matter of obtaining a long series of readings much easier than the use of the ordinary scale and telescope.

IMPROVED TYPE OF ELECTROMOTIVE THERMOMETER

In the last year the instrument has been improved in several particulars. One thermocouple only is inserted in the thermostat and thus serves as the common junction to each and every pair of thermocouples. The elimination of several thermocouples in the thermostat through the use of a common constantan-copper junction makes it possible to measure temperatures in various parts of the body when diathermy or other high-frequency apparatus is employed in treatment or observational work. From the practical standpoint, also, there is a considerable saving in the time and trouble of making and inserting in the thermostat the sixteen thermocouples which are attached in the latest form of instrument.

Another important improvement lies in the type of switch used to enable the operator to join quickly into the galvanometric circuit any thermocouple desired. The essential details of the

dials and their construction are shown in figure 7. Through the use of constantan and copper rods and ribbon (strips), it has been possible to construct a rotary switch in which all the points of contact on the upper plate (fig. 7, part 1) are of copper. A lower plate of similar construction carries constantan contacts. The copper lead wire of each thermocouple respectively is attached to the appropriate copper terminal in the upper plate, and, in turn, the constantan wires are fastened to the lower plate. The two rotating arms of the dial are made of copper (fig. 7, r_c) and con-
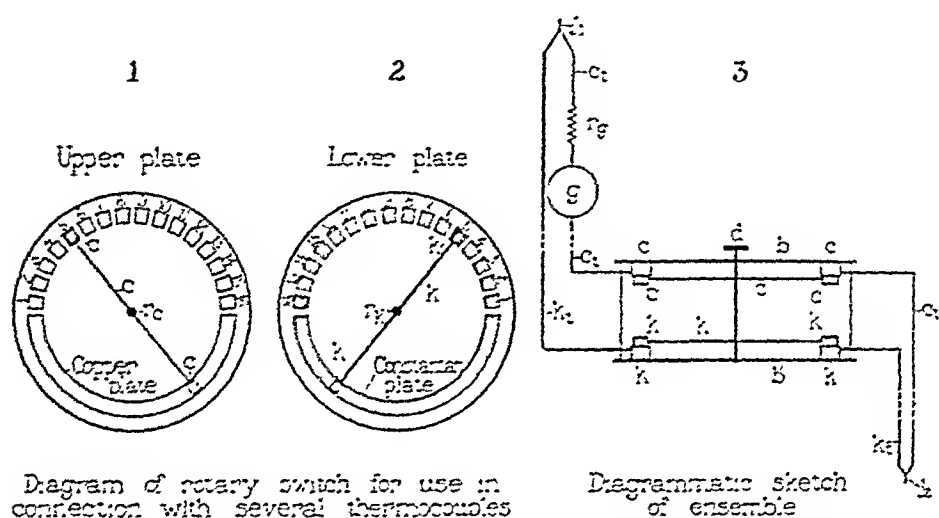
FIG. 7. DIAGRAM OF ROTARY SWITCH AND SKETCH OF ENSEMBLE

c, copper; k, constantan; r_c, rotor arm of copper: r_k, rotor arm of constantan; g, galvanometer; r_g, resistance (copper) in galvanometric circuit; b, b, supporting plates of hard rubber of bakelite, and j_1 and j_2, thermojunctions of copper and constantan.

stantan (fig. 7, r_k) respectively. The upper and lower base plates of the rotary switch are of hard rubber and bakelite. Figure 7 (part 3) shows in diagrammatic form the complete circuit when the rotary switch is set to include any desired thermocouple (for example, number 5) in the galvanometric circuit. Again, it may be pointed out that the galvanometer (fig. 7, g), the auxiliary resistance (fig. 7, r_g), the leads and the wires which form portions of one part of each complete thermocouple unit are of copper. The other portions of the circuits are constantan.

Through his careful construction of instruments, Halstead has eliminated technical difficulties and has made the electromotive thermometer a useful instrument in clinical research and in experimental medicine and biology.

APPLICATIONS OF ELECTROMOTIVE THERMOMETRY

Thermocouples inserted in surgical needles have been used by the writer and his colleagues in a study of the production of fever
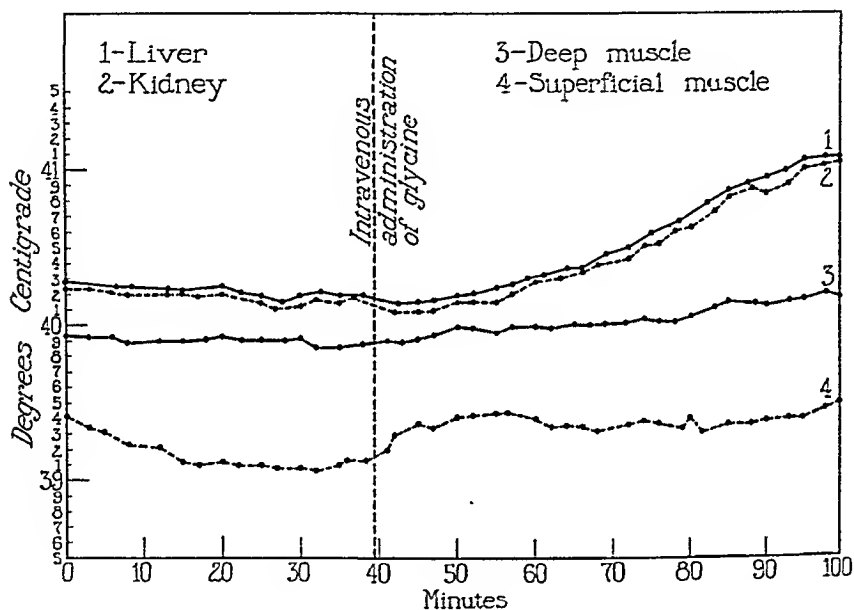
FIG. 8. RECORD OF TEMPERATURES IN THE LIVER, KIDNEY, DEEP MUSCLE AND SUPERFICIAL MUSCLE BEFORE AND AFTER THE ADMINISTRATION OF GLYCINE

in animals by various means. Figure 8 gives a series of readings of the temperatures of the liver, kidney, deep muscle and superficial muscle of an anesthetized animal before and after intravenous administration of 0.5 gm. of glycine for each kilogram of body weight. I shall not present in this article these and other data obtained in these investigations, but I have included figure 8 to indicate the adaptability of the electromotive thermometer to such experiments.

In figure 9 are plotted the readings of temperature obtained on the two great toes with cutaneous thermocouples, the gastric temperatures as obtained through the use of the special gastric thermocouple, and the oral temperature (mercurial thermometer) of a person, after the administration of typhoid vaccine (1,000,000
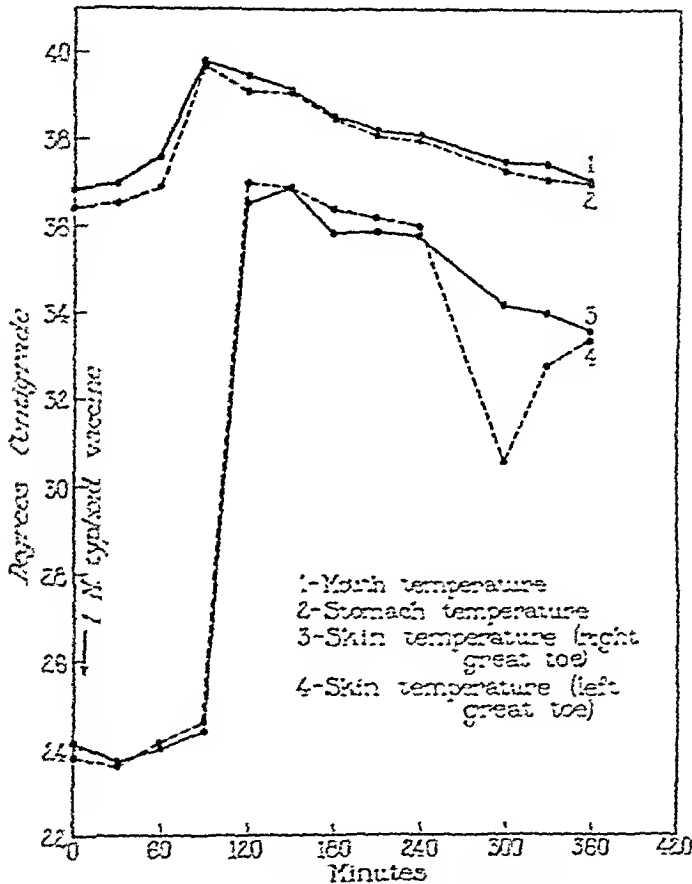
Fig. 9. Observations on the Cutaneous Temperature, Oral Temperature, and Gastric Temperature After the Administration of Typhoid Vaccine (1,000,000 *Eberthella typhi*)

Eberthella typhi). The course of the fever, as indicated by superficial and cavity temperatures, is clearly portrayed. The physiologic and clinical significance of such data is reserved for presentation elsewhere.

Extensive clinical calorimetric and thermometric studies have been made by some of my medical colleagues. In these investi-

gations they have made use of the electromotive thermometer
and the foot calorimeter previously described by me.[12]   Adson
and Brown[1] wrote:

Subjects who exhibit vasospastic disturbances are especially prone to have
decreased temperature of the skin with marked fluctuations in the involved
parts.   Under usual environmental conditions, room temperature 24° to 26°C.,
the surface temperature is low in the hands and feet, ranging from 16° to 25°C.
The surface temperature of the extremities of the average normal person varies
from 24° to 33°C.   In cases of Raynaud's disease, the fluctuations in surface
temperature are extreme and constitute an exaggerated response to variations
in the environmental temperature.   This is shown, not only with determinations
of the surface temperature by the thermocouple, but also in variations in the
rate of heat elimination as determined by the foot and hand calorimeter.   During
the stage either of pallor or of cyanosis the surface temperature of the part be-
comes excessively low and increases with recovery to normal color.   As the
disease becomes more advanced, there is an increasing tendency for the surface
temperature to remain low.   The marked vasospastic element present in these
cases also is shown in the response of surface temperature and in the rate of
heat elimination when systemic fever is induced.   For the purpose of studying
the range of the vasomotor response, a procedure has been developed which
gives information on this point and serves as a useful index in determining the
type of case amenable to operative measures.   It is particularly valuable in
cases of thrombo-angiitis obliterans which are frequently complicated by vaso-
spastic disturbances.   One of us (Brown) has devised what we call the "vaso-
motor or vascular index" which is determined as follows: Nonspecific protein
fever is induced by the intravenous injection of triple typoid vaccine, and the
surface temperatures of the digits, foot, and hand, are taken simultaneously
with the temperature in the mouth, or roughly simultaneously with the tem-
perature in the blood.   In all persons, including those who are normal and
those with or without vascular disease, after a preliminary drop due to the
chill, the temperature in the mouth and on the surface rises.   The magnitude
of the rise in the temperature of the skin is dependent on (1) the initial tempera-
ture of the extremity, (2) the severity of the febrile reaction, and (3) the patency
of the arteries.   In cases in which the extremities are cold and in cases in which
there is considerable vasospasm, the increase in the surface temperature is very
great.   The index is calculated by determining the rise in the surface tempera-
ture and subtracting from that the rise in the temperature of the mouth or blood;
this, in degrees Centigrade, constitutes the change in temperature of the skin
that is due largely to the shifting of blood that comes from vasomotor changes.
This increase, divided by the number of degrees increase in the temperature of
the blood, gives a figure which, in simple terms, indicates that for every degree
rise in the temperature of the blood there is in the temperature of the skin a

certain number of degrees of rise which is largely of vasomotor origin. In cases of Raynaud's disease, indexes of from 5 to 14 are obtained. In the cases of thrombo-angiitis obliterans with vasospastic disturbances, indexes of 2 to 6 have been found. This index is of practical importance in the selection of cases for operation, as the rise in surface temperature that comes with fever approximates roughly that occurring after a sympathetic ganglionectomy. It also has a certain diagnostic import in differentiating cases in which the diagnosis of a pure vasomotor disturbance and early organic disease of the arteries is not entirely clear. In arteriosclerotic disease of the limbs, the vasomotor indexes are low or zero. To obtain such an index militates against operation on the sympthetic system.

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A NOTE ON THE DISTILLATION OF AMMONIA IN THE FOLIN AND WU METHOD FOR THE DETERMINATION OF UREA IN BLOOD

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A simple modification of the distillation procedure in the Folin and Wu[1] method for the routine determination of urea in blood has proved extremely helpful for our hospitals. The changes proposed resulted from an attempt to overcome the trouble which some workers, particularly students and others of little experience in the laboratories, had encountered with the distillation process of the original method. The principal causes of difficulty were (1) frothing in the distillation tube with the carrying over of some of the contents, (2) sucking back of the distillate, and (3) blowing out of the distillate from the receiving tube by steam as a result of too rapid distillation. Elimination as far as possible of these potential sources of error in the original procedure has met with considerable success.

We have abandoned the use of the loosely fitting rubber stopper on the long arm of the bent glass tubing to hold the 25 cc. graduated receiving test tube* in position. Instead, we place this test tube in a 600 cc. beaker filled with cold water and bring it into place for distillation by raising the beaker so that the end of the glass tubing comes well below the surface of the 0.05 normal hydrochloric acid in the receiving test tube. The beaker is held in position during the distillation by a wooden block of suitable size.

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* A graduated test tube is used for convenience if one follows the writer's modification of the Folin-Wu method using the Peebles-Lewis Colorimeter (PEEBLES AND LEWIS, Jour. Am. Med. Assn., 70: 679, 1918; LEWIS, Jour. Lab. and Clin. Med. In press). If the original Folin-Wu procedure is to be followed, an ordinary test tube with a single graduation at 25 cc. may be used.

Very little change has been made in the technique of the method.   A piece of paraffin about three times the size of the head of an ordinary pin is used in place of the two drops of mineral oil to prevent frothing, and two small glass beads instead of a dry pebble are employed to prevent bumping.   It is important that the bent glass tubing used in the distillation apparatus should have an inside diameter of from 5 to 6 mm., as some difficulty has been
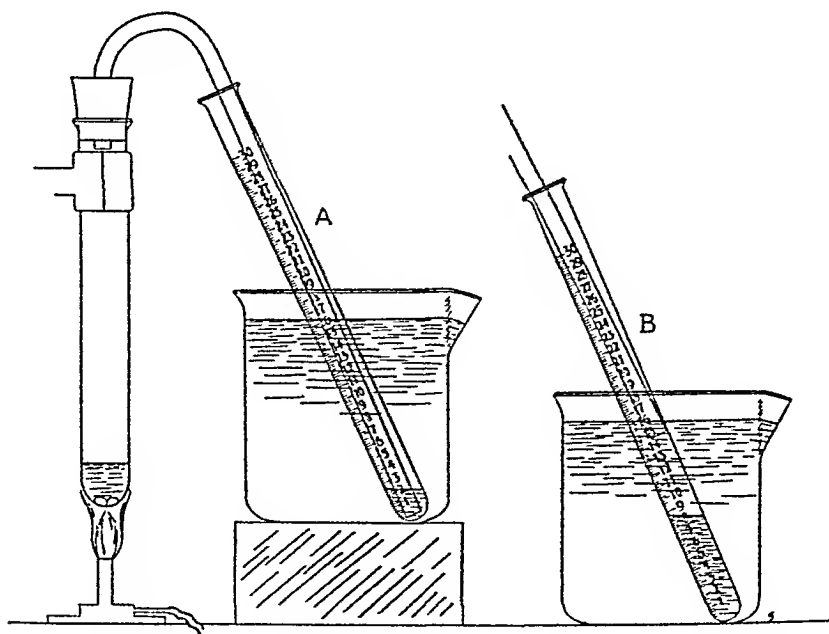

FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE MODIFIED DISTILLATION APPARATUS

A shows position of receiving tube during distillation; *B*, after completion of distillation with the receiving tube lowered by removal of the wooden block.

experienced from a tendency of the distillate to suck back when smaller tubing is used.

Distillation is started over a micro-burner and is continued until from 5 to 6 cc. of water have been distilled into the receiving tube.   Then the block is removed and the beaker is lowered to the table top, thereby dropping the graduated test tube so that the end of the glass tubing no longer dips below the surface of

the distillate. Fig. 1 is a diagrammatic illustration of the apparatus used in the distillation.

As the beaker of water keeps the lower end of the distilling tubing and the receiving test tube sufficiently cool to bring about good condensation throughout the distillation, it is not necessary to regulate the rate of distillation to prevent the emission of steam from the receiving tube before the end of three minutes. Irrespective of how rapid the rate may be, all steam will be condensed before reaching the receiving tube. No trouble whatsoever has been experienced from frothing, as mentioned by Watson and White,[2] or from bumping. There is no tendency for the distillate to suck back into the distillation tube. Consequently, after the flame is started, one may do other things about the laboratory and give absolutely no attention to the distillation until it is time to lower the receiving tube.

SUMMARY

1. Certain potential sources of error inherent in the procedure for distillation of ammonia as described by Folin and Wu in their method for the determination of urea in blood have been entirely eliminated through the changes which we have proposed.

2. The revised technique has been used in our laboratories for the past eight years with great satisfaction.

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A METHOD FOR THE DETERMINATION OF BLOOD CHLORIDES USING PALLADIOUS NITRATE AS INDICATOR*

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The use of palladious nitrate as an indicator in the titration of silver nitrate with potassium iodide in the determination of silver was introduced by Schneider[2] who stated that "the stability of palladious iodide is greater than that of ferric sulphocyanate," and that "dilution does not affect the sensitivity of the indicator." The sensitivity and permanency of this indicator suggested the possibility of its use in the determination of the relatively small amounts of chlorides contained in blood.

Numerous methods for the determination of blood chlorides have been reported in the literature. Although accurate results are possible with most of the procedures which have been proposed, the end-points of the titrations are in many instances none too sharp or, in the case of the iodometric methods, can be obtained only by the use of buffer starch indicator solutions, which do not keep well. Consequently, the introduction of palladious nitrate as an indicator in the determination of blood chlorides seemed very well worth an attempt.

PALLADIOUS NITRATE METHOD FOR BLOOD CHLORIDES

Preliminary work having shown that palladious nitrate is sufficiently sensitive to be used satisfactorily as an indicator in the

titration of silver nitrate of such low concentrations as are necessary in the determination of blood chlorides, the following method was adopted.

Solutions required

1. Sulphosalicylic acid.   A 2 per cent solution.
2. Standard silver nitrate solution (1 cc. = 1.28 mgm. NaCl).

Silver nitrate, C.P.	3.72 grams
Nitric acid, conc.	250 cc.
Water to make.	1000 cc.

3. Standard potassium iodide solution (2 cc. = 1 cc. Standard $AgNO_3$.   Transfer approximately 1.9 gm. of potassium iodide to a liter volumetric flask, dissolve in water, and dilute to volume. Measure 1 cc. of the standard silver nitrate solution, 9 cc. of water, and 0.2 cc. of palladious nitrate indicator into a small Erlenmeyer flask and titrate from a micro-burette with the potassium iodide solution to the first permanent brown color.   After checking the titration, adjust the solution by dilution so that exactly 2.03 cc. instead of 2 cc. of the potassium iodide solution will be required to titrate 1 cc. of the standard silver nitrate solution to the brown end-point with palladious nitrate indicator. The extra 0.03 cc. of potassium iodide solution is necessary to provide for the blank required to produce the end-point with the indicator.   When this standard potassium iodide solution is used in the titration of excess silver nitrate in blood chloride determinations, the same blank of 0.03 cc. is subtracted from the titration value obtained.

4. Palladious nitrate indicator solution.

Palladious nitrate.	0.13 gram
Nitric acid, conc.	16 cc.
Water to make	100 cc.

This solution keeps indefinitely.

Technique of method

To 2 cc. of plasma or whole blood in a 25 cc. volumetric flask, add about 6 cc. of water, and then 15 cc. of 2 per cent sulphosali-

cylic acid. Dilute to volume, shake, allow to stand five to ten minutes, and filter. To 10 cc. of the water-clear filtrate in a 25 cc. volumetric flask, add 5 cc. of standard silver nitrate solution, and dilute to volume. Add a small pinch of kaolin to aid the coagulation of the silver chloride formed, shake thoroughly, allow to stand five to ten minutes, and filter. If the first few drops of the filtrate are cloudy, pour back through the filter to obtain a clear solution. To 10 cc. of the filtrate in a small Erlenmeyer flask, add 0.2 cc. of palladious nitrate indicator and, using a micro-burette, titrate with standard potassium iodide to the first brown color. The end-point is very distinct and permanent.

Calculation of results

The amount of sodium chloride present may be calculated from the following formula:

$$\begin{pmatrix} \text{Milligram of} \\ \text{NaCl per 100} \\ \text{cc. of blood} \\ \text{(or plasma)} \end{pmatrix} = \left\{ 2 - \frac{\text{KI used} - \text{titration blank}}{2} \right\} \times \begin{pmatrix} \text{mgm. of} \\ \text{NaCl per} \\ \text{cc. of} \\ \text{AgNO}_3 \end{pmatrix} \times \begin{pmatrix} \dfrac{100}{\text{Blood (or plasma)}} \\ \text{equivalent of} \\ \text{filtrate used} \end{pmatrix}$$

$$= \frac{4 - (\text{KI used} - 0.03)}{2} \times 1.28 \times \frac{100}{0.32},$$

$$= 4 - (\text{KI used} - 0.03) \times 200.$$

Thus, to find the number of milligrams of sodium chloride in 100 cc. of plasma (or whole blood), subtract 0.03 (the titration blank) from the number of cubic centimeters of KI used, then subtract this figure from 4, and multiply the difference obtained by 200.

Accuracy of method

Proof of the accuracy of the proposed method and of the sensitivity of palladious nitrate as an indicator is given by the results obtained when varying amounts of hydrochloric acid of known normality were used in place of blood filtrate and their chloride contents were determined according to the latter part of the method as outlined. Table 1 shows that the amount of chloride found in each case corresponds closely to that actually present.

TABLE 1

COMPARISON OF THE THEORETICAL CHLORIDE CONTENTS OF VARYING AMOUNTS OF STANDARDIZED HYDROCHLORIC ACID SOLUTIONS WITH THOSE FOUND BY THE PALLADIOUS NITRATE METHOD

HYDROCHLORIC ACID (1 cc. = 0.559 mgm. NaCl)	CHLORIDE AS NaCl	
	Theoretical	Found*
cc.	mgm.	mgm.
9.0	5.03	4.99
9.1	5.09	5.07
9.2	5.14	5.12
9.3	5.20	5.19
9.4	5.26	5.22
9.5	5.31	5.27
9.6	5.37	5.36
9.7	5.42	5.43
9.8	5.48	5.46
9.9	5.54	5.50
10.0	5.59	5.57

* Average of duplicate determinations.

TABLE 2

COMPARISON OF PLASMA CHLORIDE VALUES OBTAINED BY THE MCLEAN-VAN SLYKE METHOD AND THE PALLADIOUS NITRATE METHOD

SAMPLE NUMBER	NaCl PER 100 CC. BLOOD PLASMA*	
	McLean-Van Slyke method	Palladious nitrate
	mgm.	mgm.
1	640	644
2	596	590
3	556	559
4	596	590
5	611	608
6	554	548
7	596	588
8	550	547
9	541	550
10	635	634
11	571	578
12	604	597
13	589	586
14	587	580

* Results with both methods are averages of duplicate determinations.

Table 2 demonstrates even more convincingly that the method is accurate for the determination of the chlorides of blood, as it shows, by typical examples, the close agreement between the plasma chloride figures obtained by the proposed method and those secured with the procedure of McLean and Van Slyke.[1] Inasmuch as these investigators obtained, by their method, values which agreed within 0.51 per cent with those found by fusion, we may assume that our method gives an accurate estimate of the true chloride content of plasma.

Finally, attention should be called to the fact that, according to our experience, the chloride content of whole blood may be determined by this method with the same degree of accuracy as has been demonstrated for plasma.

CONCLUSION

Our desire to secure a simple indicator, which would give a sharper and more permanent titration end-point than it is possible to obtain with sulphocyanate and ferric alum, has been more than satisfied by the introduction into blood chemistry of palladious nitrate for the titration of silver nitrate with potassium iodide. This indicator requires no specific buffering and gives a sharp, clear, easily recognizable end-point lasting at least over night.

SUMMARY

1. A method has been proposed for the determination of the chlorides of whole blood or plasma.

2. By introducing sulphosalicylic acid as the protein precipitating agent and palladious nitrate as the indicator in titrating excess silver nitrate with potassium iodide, a rapid, accurate procedure has been developed.

3. The end-point is sharp, clear, permanent and easily recognizable.

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THE PRESENT STATUS OF CLINICAL LABORATORY MEASUREMENTS WITH A NOTE ON THE PHOTO-ELECTRIC EFFECT*

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GENERAL CONSIDERATIONS

Excepting only the descriptive, it is generally recognized that every branch of science is fundamentally quantitative and that improvements in measurements are necessary for scientific progress. Thus the problems of the pure sciences are often wholly matters of measurement while those of the applied sciences are inextricably bound up with the very concrete matter of efficiency of production. Workers in the pure and applied sciences are, therefore, always keenly aware of the importance of improving methods of measurement.

That physicians do not so clearly comprehend the nature and importance of measurements is evident in many ways. Thus a recent issue of a first rate medical journal contained, among others, two papers, one of which gave data to show that protein fractionations could be made more precisely by interferometry than by refractometry, while the other gave clinical data of cases in which proteinuria was a prominent characteristic, designating the protein concentrations by one or more crosses. Evidently a forward looking editor saw nothing incongruous in presenting at the same time methods for estimating the same material which in one instance are of extreme refinement and in the other of extreme crudity. Such instances are not at all unusual; on the contrary, practically *every text on clinical pathology has numerous ex-*

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amples of indiscrimination between qualitative, semi-qualitative and quantitative methods.

Perhaps the dual conception of the practice of medicine as being partly art and partly science accounts for the inchoate appreciation of the nature and value of precise measurements, because duality naturally tends to confuse and the confusion seems to make it difficult for many physicians to distinguish clearly between what is art and what is science in medicine. However that may be, it is, nevertheless, certain that the art of diagnosis only begins and can only function to best advantage after science has developed as accurate and complete information as possible about a case. Physicians who are satisfied with anything less than the best information that science can give them about their cases, therefore, fail to do justice to themselves, their patients and their art.

Of the tests most commonly applied in practice to develop clinical information, those for protein, sugar and the formed elements in urine are good examples of the illusory habit of trying to draw quantitative deductions from purely qualitative tests, which still persists among physicians who have not yet learned that the newer quanitative methods, at no more cost in time and trouble, supply much more useful information than the older hit-or-miss qualitative methods.

Furthermore, those clinicians who follow the trend of modern experimental medicine already insist on knowing not its mere concentration but the rate at which patients excrete substances of particular interest, because such information not only has much more definition and clinical value but is unquestionably the truest criterion for comparing samples excreted at different times by the same individual. Being a simple correlation of time, volume and concentration, the rate of excretion, of course, depends upon accurate quantitations of the excreted substances, and the responsibility of providing better quantitative information rests squarely upon clinical pathologists.

All good physicians aim at earlier and earlier diagnosis, but advancement in this extremely important field is hindered by the insufficient sensitivity of many of the tests in common use. Of

these the tests for indican and ketones in urine may be taken as examples, and here again clinical pathology has the obligation to provide not only more accurate but also more definite earlier information.

Again, there are certain kinds of very valuable information which are not available in practice as early and as generally as they should be because their technics are somewhat complicated and time-consuming. Blood cholesterol, calcium, non-protein nitrogen and urinary ammonia are examples of this kind of information; and for the good of the public it is, of course, desirable that such technics be brought within the realm of clinical routine.

If there is any branch of medicine which is essentially scientific and therefore quantitative, it is that which has come to be known as clinical pathology, and it is the function of the clinical pathologist not only to provide complete and exact laboratory information but also to interpret such information and advise the kinds of information which may or may not be useful or suggestive in a particular case. Clinical pathologists should, therefore, be familiar with the newly won facts of experimental medicine because that special knowledge, hand in hand with the ability to perform more complicated procedures like sulfur partition or protein fractionation, may often lead to clearing up obscurities which surround doubtful cases.

There are other considerations which also show how vital to the practice of medicine is the advancement of clinical pathology along lines which broaden its scope and refine its technics so as to make them more precise and practical and therefore more generally useful. Inevitably every improvement in the accuracy and sensitivity of clinical pathological technics registers an increase in clinical abilities. Thus the progress of medical science depends upon improving methods of measurement.

Measurement of the many materials of clinical interest in the various body fluids usually involves both chemistry and physics. The chemistry has, of course, been contributed by painstaking pioneer work of the chemists who, unfortunately, have not been accustomed to restricting bench manipulations to a minimum or to dealing with limited amounts of material. Thus the litera-

ture of biochemical technics is rich in excellent methods which have never passed beyond the status of mere records and remain clinically useless because too complicated and time consuming. In fact, it has been the history of many of our most valuable clinical methods that after publication the technics undergo a series of modifications and simplifications extending often over years before they become generally useful for clinical routine. Undoubtedly the demands of science for precision and the demands of economics for simplicity and rapidity are somewhat conflicting, but the many successful reconciliations which have already been accomplished point unmistakably to the way of improving the practice of medicine.

Outside the laboratory when information is immediately needed, as for instance in the operating room or at the bedside, a reasonable compromise between speed and accuracy is expedient, but conceding accuracy to convenience in the laboratory is never justifiable unless the resulting effects have been rigorously considered and are clearly understood because every loss in accuracy is a loss in scientific ability. That this situation is clearly understood by many clinical pathologists is evident from the continual search for better and more precise methods. That others do not appreciate it is evident from their willingness to employ inferior methods, usually because they have not progressed beyond a qualitative state.

HEMOGLOBINOMETRY

Its general application and great clinical importance account for the fact that hemoglobinometry is performed oftener than any other blood test. It also happens that during the past few years in journals and meetings both clinical pathologists and clinicians have expressed marked dissatisfaction with current hemoglobin methods. Hemoglobinometry, therefore, appropriately illustrates the present status of colorimetry in clinical laboratories.

Van Slyke's oxygen capacity and iron determinations are admittedly the best of all hemoglobin methods. While exceedingly valuable for standardizing purposes and for checking other methods, they are, unfortunately, too exacting for clinical work.

Of the methods practically applied, some crudely measure the colors of a stain or smear of native blood.   The other methods by more or less quick and simple manipulation convert hemoglobin into oxy-hemoglobin or the more stable acid hematin.   All of them compare the color of the sample with some kind of standard by means of some optical device.

Not one of the methods has won its way into general use, and all of them have been criticized either as to accuracy or practicability.   The existing situation may, therefore, be described as one in which many different methods giving inaccurate and different kinds of results are now being used in our clinical laboratories.

If some kind of animal or other source of supply of a stable hemoglobin solution containing say 15 grams per 100 cc. were conjured up, all of the perplexities and difficulties that now beset hemoglobinometry would quickly disappear because such a mythical standard would enable a clinical pathologist to determine hemoglobin with whatever precision he desired.   Thus, one could settle on a weak looking standard and dilute the sample until the colors looked alike, or one might make different dilutions of the imaginary standard so as to have a series of concentrations in test tubes to slap against and compare with the sample in some sort of perforated box.

On the other hand, a clinical pathologist intent on developing the kind of information which can be relied upon for close diagnostic reasoning, to tell if a patient is anemic or not in borderline cases, to detect and help follow the course of hemorrhage, to enable him to appraise the effects of treatment, or to furnish data for correct statistics, could secure the necessary precision by using an accurate photometer like a well made Duboscq colorimeter. On final analysis, then, it is clear that the absence of standardizing difficulties resolves the matter of precision into a mere choice of measuring device.

Unfortunately, our imaginary standard is only one of many ideal standards for measuring biologic materials which are also lacking.   In the case of hemoglobin, some supply the deficiency by meticulously preparing standards from chance specimens of

blood subjected to careful oxygen capacity or iron determination. Although theoretically and practically correct, the preparation and maintenance of such standards are impossible in many clinical laboratories.   It therefore happens that much ingenuity has been expended in attempts to secure satisfactory substitutes. These usually take the form of colored liquids or glasses.

The liquid standards are more or less stable solutions of inorganic materials or dyes.   Their great deficiency, however, is that which they have in common with the colored glasses, and this defect is well illustrated by Newcomer's[2] experience.

As a result of his painstaking search for a standard suitable for use in place of a certain definitely prepared concentration of acid hematin, Newcomer found a substitute in the form of a disc cut from a particular melt of glass which happened to have a spectral transmission similar to that of his acid hematin.   The Duboscq colorimeter was adapted for the disc and the manufacturer supplied standards cut from the original batch of glass as long as it lasted.   The original Newcomer method is now necessarily limited to those fortunate enough to have one of the standard discs because the most expert technologists have never been able to reproduce the glass.   This is all the more regrettable because none of the currently used glass hemoglobin standards have the same spectral transmissions as the samples and Newcomer's is the only hemoglobin method employing a glass standard which is based upon correct scientific principles.

In the early days of clinical colorimetry when the underlying optics were not so well understood by chemists and clinical pathologists many attempts to avoid the standardizing troubles of Duboscq colorimetry were made by employing solutions of dyes or inorganic materials.   Experience, however, soon taught that Duboscq colorimetry is inaccurate when the spectral transmissions of standard and sample are not the same.   Such colored liquids, like the colored glasses, are therefore unfit for critical comparisons with the samples they are supposed to measure, and the present unsatisfactory state of hemoglobinometry and other quantitations by colorimetry must be laid to the extensive use of standards which only roughly resemble and cannot be critically compared with the unknown sample.

JUNIOR SCOPOMETRY

Owing to the nature of dispersed materials the standardizing troubles of nephelometry and comparison turbidimetry are even greater than those of colorimetry and they account for the failure of these highly sensitive and potentially very valuable methods to give better results than the ancient disappearance or extinction criterion which needs no standards at all.   To exploit this feature to the utmost advantage, the disappearance criterion was refined and improved and as made available in the Junior Scopometer[1] measures turbidity with greater accuracy than other clinical methods and with even less time or trouble than the crudest of existing devices.

As a result of efforts to obtain similar advantages in colorimetry a novel extinction method of colorimetry was later incorporated in the Junior Scopometer which frees hemoglobinometry and other colorimetric methods of standardizing difficulties by substituting for the usual standards reference graphs or tables showing the equivalents of concentrations and scale readings.

In the case of hemoglobin, for instance, such a calibration is made by determining the hemoglobin content of a sample of blood known to be higher than normal by the exacting oxygen capacity or iron method.   Other parts of the same sample, diluted so as to have a series of concentrations covering the desired range, are subjected to some pre-determined acid hematin or oxy-hemoglobin method and the disappearance points then measured in the Junior Scopometer.   By plotting the known concentrations against the Scopometer scale readings a calibration is obtained which thereafter need never be made again because the scale readings obtained by similarly treating subsequent samples are always referable to the original calibration.

While the Junior Scopometer is more generally applicable and gives more uniform and accurate results than the so-called permanent standard methods (i.e., colored glasses or liquids in tubes) there are certain disadvantages inherent in the physiological optics of its extinction criterion which depend upon the fact that the human eye cannot mark the disappearance of an object as critically as it can compare the brightness (color) of two specimens

with the aid of such refined optical means as a good photometer of the Duboscq type.

NOTES ON THE PHOTO-ELECTRIC EFFECT

The variabilities and limitations of human vision are such that physicists have always dreamed of emancipating optical measurements from the bias of personal equation, and a promise of the dream coming true was made almost half a century ago by the discovery of the photo-electric effect which transforms light into electricity by means of photo-electric cells. These take the form of glass bulbs containing two electrodes; one, the cathode, is light sensitive and emits electrons when exposed to light; the other, or anode, when subjected to positive voltage attracts and gives direction to the electrons leaving the cathode. An efficient photo-electric cell is, therefore, a valve emitting an electric current which is linearly proportional to the stimulus of incident light.

That the earliest experimenters applied such a device to photometry is not surprising, and, as a matter of fact, photo-electric photometers were constructed and used by physicists within a year or two after Hertz's discovery. The early instruments measured the minute currents emanating from the cells with the most sensitive of known devices such as electroscopes and electrometers, and this, with other practical difficulties, necessarily restricted their operation to experimental physicists. Following the early experimental work it is interesting to note that while the theoretical literature of photo-electricity was constantly growing practical attempts to apply photo-electricity to photometry remained at a standstill until the invention of the thermionic amplifying tube by De Forest.

As it conferred other abilities as well as enabling measurements of the minute photo-electric currents, amplification naturally stimulated a renewed and enthusiastic interest in the practical possibilities of photo-electricity which have since been realized and brought to the public notice in the form of talking movies, counting, sorting, smoke recording and advertising devices, television, etc.

Amplification also made photo-electric star, lamp and spectro-

photometry practicable and has since been applied in many devices designed to improve other photometric procedures such as colorimetry and turbidimetry. Of these it appears that many have never been carried beyond the stage of design, that some were built for frankly experimental purposes, and that others, like Sheard and Sanford's[3] photo-electrometer, are satisfactory in the hands of their designers.

The photo-electric effect, or electric eye, as it has been aptly called, being far superior to human vision in its ability to mark the degree of brightness without a comparison standard, offers the possibility of combining in one method all of the advantages and conveniences of Junior Scopometry with as good or better accuracy than that of the best visual photometry. That such an instrument would be ideal for clinical laboratories is, of course, self-evident, and the fact that none is even now generally available must be laid to difficulties connected with the practical application of photo-electricity.

This becomes immediately apparent to any one who experiments with existing or proposed devices because all of the available amplifying arrangements require at least five different unvarying intensities of electricity (light, plate voltage, thermionic tube elements). These are usually supplied by different kinds of batteries which necessitate both permanent and temporary connections in addition to the variable resistances, potentiometers, and other meters and accessories needed for adjustments that are necessary to maintain calibration. Besides these there are other variables which make amplification devices hopelessly impracticable for any but the exceptional clinical laboratory because the conditions under which measurements are made in clinical laboratories demand instruments having the utmost simplicity of design, rigidity of construction, the fewest possible adjustments, and the highest degree of stability with the greatest possible convenience of operation.

In attempts to apply the electric eye to Scopometry I tested out the possibilities of various glow tubes and other amplifying devices with different circuits, and in the course of these experiments was able to secure a number of different ar-

rangements which functioned successfully when continuously watched, adjusted and checked with meticulous care. In fact, under such conditions an astounding degree of sensitivity was obtained with several setups. Because of the intricacies and complications, however, which seem to be inseparable from present methods of amplification none of them, by any stretch of one's imagination, could be considered practicable and safe enough to put into the hands of the usual run of laboratory technicians.

Believing it essential that an instrument intended for clinical laboratory measurements must be as nearly fool proof as possible, I then explored other approaches to the problem which were free from the need of amplification and supersensitive electrical measuring devices. As a result of these experiments I finally secured a setup which has proved so satisfactory that it is still in routine use in the Prudential Laboratory. From figure 1 it will be seen that a beam of light taken from an incandescent lamp is split into two by means of a partly transparent and partly mirrored glass. One of these optical paths goes straight through transparent stripes of glass to a photo-electric cell, while the other is reflected by mirrored stripes to a similar photo-electric cell. The cells are connected with a rugged short period galvanometer in a simple Wheatstone bridge circuit so that the currents from the photo-electric cells balance one another when they are equal. For balancing the light traversing the two optical paths there is a provision in one for interposing the specimen to be measured and in the other for a diaphragm whose aperture can be increased or diminished at will. Thus a specimen placed in one path is measured by simply turning a pinion which changes the aperture of the diaphragm in the other path until the galvanometer registers a perfect balance by pointing to zero. At this point a reading of the scale attached to the diaphragm gives the measure of the specimen when referred to a predetermined calibration like those of the Junior Scopometer.

Such a calibration for hemoglobin (acid hematin) is seen in figure 2 which also indicates the unusual sensitivity of the method without the light filters used in Junior Scopometer colorimetry.

Figure 3 shows the calibration of a graduated series of sodium picramate solutions whose run of brownish red colors are similar to those of the di-nitro salicyclic and picric acid sugar reduction methods.
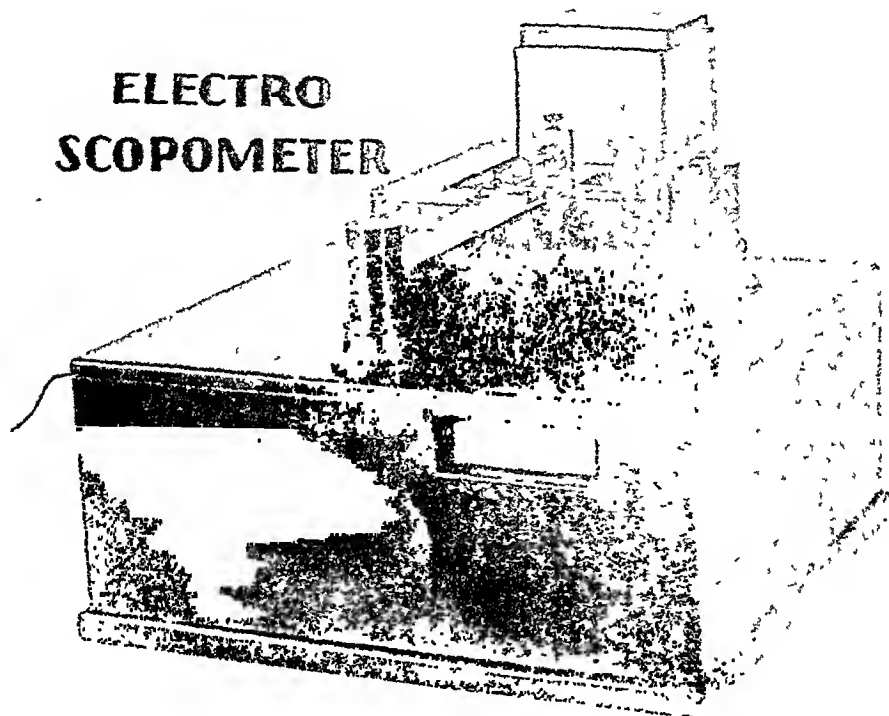

Fig. 1. Experimental Photo-electric Setup with Provisions for Measuring Specimens by Either Reflected (Left) or Transmitted Light (Center)

Adjustable diaphragm with scale on right. Note the single electrical connection for house current. In its finished form this device will be known as the Electro Scopometer.

Figure 4 gives a calibration for measuring blue solutions like those obtained with the sugar, phenol, uric acid and other molybdic reduction methods of Folin and Benedict.

Figure 5 indicates the extraordinary sensitivity with which the method measures turbidities by transmitted light as shown by the calibration for counting red blood cells.

Figure 6 shows the results of two experiments made on the same material. One of them, to determine the stability of a particular suspension, was made by precipitating a graduated series of serum
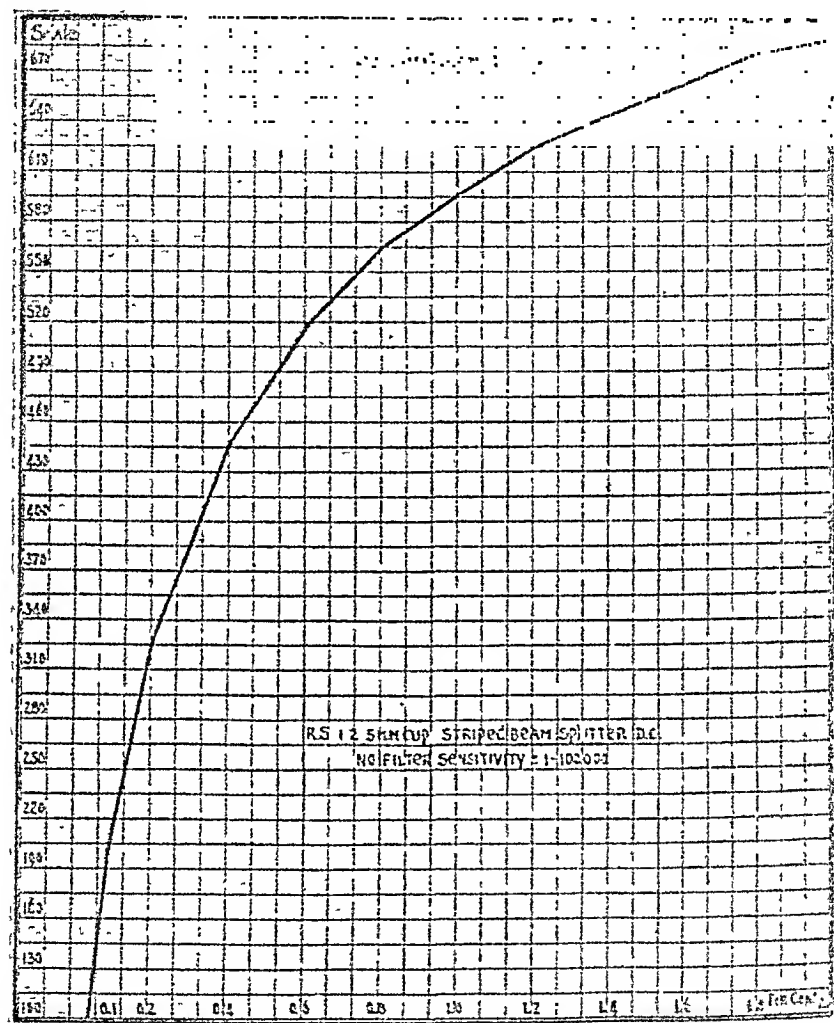
Fig. 3

protein concentrations with Exton's reagent and plotting the concentrations against the scale readings obtained with the instrument and then re-reading the suspensions after twenty-four hours standing. The results of this experiment indicate remarkable

FIG. 2

FIG. 4

FIG. 5

FIG. 6. Stability experiment indicated by lines. Precision experiment indicated by circles.

stability of suspensions obtained in this way. The second experiment was designed to indicate the precision of the method and was carried out by giving three individuals who had never seen the instrument a few minutes instruction and then plotting their single readings also on the graph. It will be noted that the differences are less than 1 per cent. In this connection it is also of interest to note that the precision of the instrument taught us the necessity of refining some bench manipulations which were hitherto regarded as satisfactory (i.e., with visual instruments).

In conclusion it remains to be said that only those who have enjoyed the opportunity can adequately appreciate the ease with which accurate measurements are made possible by the photoelectric effect.

SUMMARY

The present status of clinical pathology is discussed with special reference to improving its usefulness by more informative and precise methods than are now generally employed, taking hemoglobinometry as an illustration of the fundamental need for improving methods of measurements.

The advantages of the photo-electric effect over visual methods are discussed and experiments in trying to overcome difficulties connected with the practical application of photo-electricity to the requirements of routine clinical pathology are cited.

An experimental photo-electric setup which has proven decidedly satisfactory in routine work on account of its stability, rapidity and ease of operation is described and its universal applicability illustrated by calibrations which also indicate the extraordinary range, sensitivity and accuracy of the method.

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EXPERIMENTAL FAT NECROSIS IN VARIOUS VERTEBRATES*

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Langerhans[2] injected an extract prepared from the pancreas of rabbits into nine rabbits and three dogs, and obtained in one of the rabbits the first experimental fat necrosis. Except for the small degree of success, numerically speaking, and for the small number of animals used in the experiment, in view of the fact that negative results were obtained in the dogs, a conclusion might have been drawn that the extract was not potent, or functional in the sense of its ability to produce fat necrosis in heterogeneous species.

Jung,[1] by placing pieces of dog's pancreas in the abdominal cavity of rabbits, and Wells,[5] by injecting cats, dogs and rabbits with an extract prepared from fresh pancreas of the hog, produced fat necrosis. The results of these two investigators proved that the fat necrosis producing substance was not, therefore, one limited to homogeneous species.

The work here recorded was taken up with the intent to add further proof to the recorded facts as to the non-homogeneous specificity of the fat necrosis producing substance, and also to determine the response in different types of vertebrates to the action of this particular ferment or enzyme on their fat deposits. This diversity of species brings in the questions of species specificity, of variation in the response of the fat deposits in different species, of the hydrogen ion values for the different vertebrates, and of the body temperature as possible factors influencing the experimental production of fat necrosis.

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EXPERIMENTAL PROCEDURES

The vertebrates used fall into two main groups,—the cold-blooded and the warm-blooded varieties. In the cold-blooded group, there have been used: *Lepisosteus platystomus*, the short-nosed gar, *Cyprinus carpio*, the German carp, and *Macroclamys pseudo-geographica*, and *Chrysmes belli cinerea*, two types of turtles. The cold-blooded animals were kept in aquaria with a rather constant temperature of 18° to 20°C. Two groups of warm-blooded vertebrates were used. One, the mammalian group, included *Mus decumanus*, the white rat, and *Canis famil-*

TABLE 1

SUMMARY OF POSITIVE RESULTS OBTAINED BY SUBSTANCES EMPLOYED IN THE EXPERIMENTAL PRODUCTION OF FAT NECROSIS

SPECIES OR TYPE ANIMAL	SUBSTANCE INJECTED							
	Commercial pancreatin	Fresh hog's pancreas	Dog's pancreatic secretion	Lipase fractions derived from:				
				Commercial pancreatin	Hog's pancreas	Peanuts	Sunflower seed	Castor bean
Fish...............	+	0	0	0	0	0	0	0
Turtles.............	+	0	+	0	0	+	0	0
Hens...............	+	0	0	0	0	+	0	0
Pigeons.............	+	0	0	0	0	0	0	0
Rats...............	+	+	+	+	+	+	+	+
Dogs...............	+	0	0	0	0	0	0	0

+ = positive; 0 = no test.

iaris, the common dog, having a normal temperature of around 37°C. In the other, the avian group, there were used *Gallina domestica*, the domestic hen, and *Columba livia*, the street pigeon, for which the respective normal body temperatures are given[4] as 40.5° to 42°C., and 41° to 43°C.

The investigations are based entirely on intraperitoneal injections into fish, turtles, hens, pigeons, rats and dogs, of various substances containing the fat necrosis producing enzyme, lipase.[3] The primary aim has been to determine the ability of the various extractives to produce fat necrosis in this varied group of verte-

brates.   These records include only those animals that survived injections long enough or had doses sufficiently large to develop lesions of fat necrosis.

Injections into fish

Injections of pancreatin (Armour's lot No. 205316) into German carp and gar resulted in the production in twenty-five hours of fat necrosis in one carp out of four that were injected. and in one gar (fig. 1) out of six.   This low percentage of positive results was to a certain degree dependent upon the fact that soon after

Fig. 1. Gross Lesions of Fat Necrosis Twenty-five Hours after Injection of Armour's Pancreatin.   Gar No. 6

being received and injected. most of the fish died, probably as a result of trauma received in shipping.

Injections into turtles

Emulsion of pancreatin (Armour's lot No. 205316) was injected into seven turtles, and of these, four developed fat necrosis, as demonstrated at forty-seven and ninety-six hours, and six days. Three turtles yielded negative findings at from seventy to one hundred and twenty hours.

Pancreatic secretion obtained from three dogs through canula

placed into the pancreatic duct, pooled, and then injected into four turtles, yielded two positive results at the periods of sixty-five and one hundred and twenty hours (figs. 2 and 3). Two turtles receiving like doses of dog's pancreatic secretion gave negative findings at fifteen hours and sixteen days.

Fig. 2. Gross Lesions of Fat Necrosis Sixty-five Hours after Injection of Pancreatic Secretion from Dog, (enlarged ×11). Turtle No. 13

The lipase fraction, "arachis concentrate" derived from peanuts and injected into five turtles, resulted in fat necrosis in two of them at ninety-six hours. Three others showed no fat necrosis in its usual sense, but did reveal marked changes in the contained eggs at seventy-two and ninety-six hour observations.

All turtles were killed at the stated time periods.   There were no fatalities, hence no possibility for post-mortem degenerative changes to play a rôle in the recorded observations.

FIG. 3. GROSS LESIONS OF FAT NECROSIS 120 HOURS AFTER INJECTION OF PANCREATIC SECRETION FROM DOG.   TURTLE NO. 14

Injections into hens

Emulsion of pancreatin (Armour's lot No. 205316) injected into three adult hens, yielded a positive result when the hen was

killed at the end of the seventy-hour period. A second hen died from hemorrhage within two hours, and the third was dead and partially decomposed at nineteen hours.

Arachis concentrate injeeted into three hens produced at twenty and forty-six hours, positive results in two, but at the end of seventy hours, no lesion was found in one animal.

Injections into pigeons

Emulsion of pancreatin (Armour's lot No. 205257) was used in the injection of eleven pigeons. Of this number, six gave positive results for fat necrosis at periods of from five hours to eight days. Five pigeons received similar doses of pancreatin emulsion but failed to show fat necrosis at periods of time ranging from four to seventy-five hours.

Injections into rats

Emulsions of pancreatin (Armour's lots Nos. 205257 and 205316) were injected into forty-nine white rats. Of this group, forty developed lesions of fat necrosis as demonstrated at from three hours (histologic findings only at this time) after injections up to and including thirteen days. Two rats gave negative findings on the seventeenth day after injection, as did three on the twenty-first day. The absence of findings after the thirteenth day indicated healing had taken place, for the lesions found in two rats at that time were histologically almost healed processes.

Two rats were given a solution prepared from macerated fresh pancreas of the hog, and showed fat necrosis at sixteen and one-half hours. Injections into ten rats of the lipase fraction from hog's pancreas produced positive results in six (fig. 4) at from twenty-one to ninety hours. Four rats that also received preparations of extract of hog's pancreas gave negative findings at from six to seventy-two hours.

Five rats were given pancreatic secretion pooled from three canulized dogs. Of these, three showed fat necrosis at from fifteen to ninety-six hours, whereas two showed no such lesions at fifteen hours.

Extracts from sunflower seed (*Helianthus annuus*) refined down to the fraction now recognized as lipase carrying, when injected into three rats gave in one at seventy-two hours, many foci of macroscopic and microscopic fat necrosis. The other two which died between six and twenty-one hours, were negative for these lesions.

Two different preparations from peanuts (*Arachis hypogaea*) were used in injections of white rats. One was termed "arachis

FIG. 4. HISTOLOGIC PREPARATION OF FAT NECROSIS SEVENTY-TWO HOURS AFTER INJECTION OF LIPASE FRACTION, ACID SERIES, FROM HOG'S PANCREAS (ENLARGED ×80). RAT NO. 56

alba," and the second "arachis concentrate," a brown mixture representing a more highly refined and concentrated extract of the lipase. Injections into twenty-seven rats of these products obtained from peanuts, gave fat necrosis in seventeen. Of the fifteen receiving arachis alba, seven showed fat necrosis at from seventy-one hours to eight days. Observations on eight showing no fat necrosis were made at periods of from twenty hours to five days. Ten of the twelve rats receiving arachis concentrate

developed fat necrosis at intervals of from six hours to five days. The other two rats gave negative findings at seventy hours and five days.

Injections into dogs

Two dogs (fig. 5) each injected with emulsion of pancreatin (Armour's lot No. 205316) and killed ninety-three hours later, showed marked fat necrosis in all intra-abdominal fat deposits.
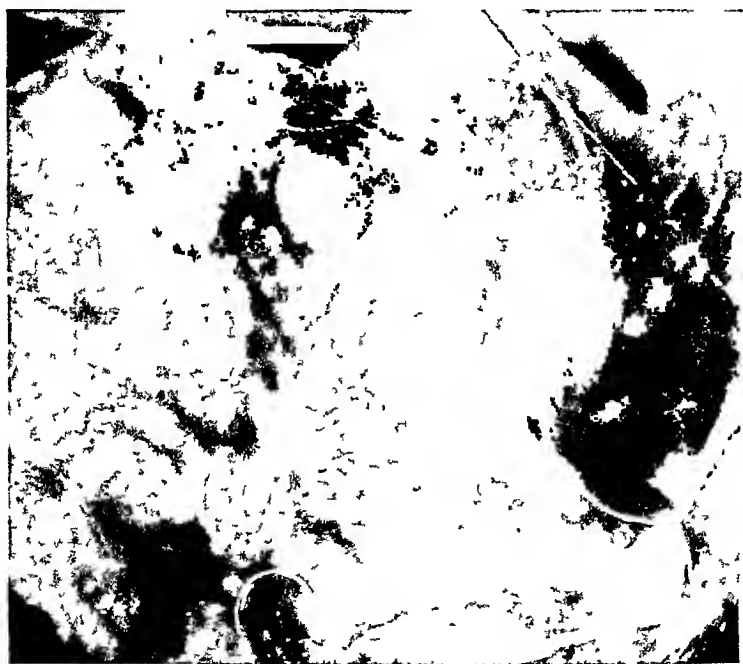
These results are summarized in the tabulation.

Fig 5. Gross Lesions of Fat Necrosis in Omentum Ninety-three Hours After Injection of Armour's Pancreatin. Dog No 2

FACTORS OF SPECIES DIFFERENCES

Species specificity

Certain of the vertebrates, even species of vertebrates, are immune or highly resistant to some of the common diseases to which man or other vertebrates are subject. Some of these species resistances can be accounted for by the factor of normal

or variable body temperatures, for example, Pasteur's well-known experience with anthrax in fowls where body temperatures were lowered. Some of the individual and species immunity, or lack of susceptibility as it appears to be in some cases, cannot with our present knowledge be measured or determined except by direct exposure to the factor or factors that are known commonly to produce the series of developments or lesions in others. Insofar as fish (carp and gar), turtles, chickens, pigeons, white rats, and dogs are concerned, there is no such species resistance against the specific action of lipase in its production of fat necrosis.

Differences in the fat of cold-blooded and warm-blooded vertebrates

In the warm-blooded species, as a part of the body metabolism, fat is stored in various locations: these storage centers or supplies are drawn upon for heating the body, and also act as contributors in the maintenance of general body energy. In the cold-blooded types, the fat deposits or depots are somewhat different from those of the warm-blooded species, and are subsidiary to the reproductive activities of the individual. In the pre-reproductive stage of the cold-blooded vertebrates, fat deposits are most in evidence. As the reproductive stages mature, the fat deposits decrease in amount, and often disappear.

In the turtle, some of the fat bodies are of a very primitive, embryonic type. Grossly they appear as a lymphoid-adipose tissue.

It was found that fat necrosis could be produced equally well in two species of cold-blooded vertebrates, fish and turtles, as well as in warm-blooded types, hens, pigeons, rats, and dogs. The type of fat, that is, whether purely energy and heat-producing stored deposits, or that type stored for the part it plays in the reproductive cycle and activity, does not interfere with the ability of lipase to produce fat necrosis in these particular fat bodies.

Hydrogen ion concentration values of the vertebrate fluids as a factor in the production of fat necrosis

The pH values of normal blood serum for the vertebrates in which fat necrosis was produced were: rat, 7.3; dog, 7.3 to 7.5; turtles, 7.5; pigeon, 7.5; carp, 7.6; gar, 7.6; and hen, 7.6. to 7.7,

or a maximal difference of from 7.3 to 7.7. This variation of 0.4 in pH value, or the buffer value indicated thereby, played no part, nor modified in any way, the production of fat necrosis in the work on the vertebrates here recorded.

Body temperature as a factor in the production of fat necrosis

Three distinct groups of vertebrates depending upon their normal body temperatures were used—first, fish and turtles, poikilothermic animals, second, mammalian types, rats and dogs, and third, avian types, hens and pigeons.

In these experiments, fat necrosis was produced in cold-blooded and warm-blooded groups at several temperatures between 18° and 43°C. Insofar as our data go, there is a suggestion that the necrosis developed more slowly in the cold-blooded animals.

ACTIVE PRINCIPLE CONCERNED IN THE PRODUCTION OF FAT NECROSIS

In 1929, we reported[3] having isolated by accredited chemical procedures, a lipase fraction from: (1) fresh pancreas of the hog; (2) commercial pancreatin, and (3) the dried seeds of: (a) Arachis. the peanut; (b) Helianthus, the common sunflower, and (c) Ricinus communis, the castor bean. The lipase fractions from each of these sources have consistently given typical fat necrosis when injected into certain vertebrates.

Regardless of the general source from which this isolated fraction was obtained, and irrespective of which type or species of the above recorded vertebrates was used, fat necrosis was produced in all alike. From a biological standpoint this clearly speaks in favor of the active substance being classed as a ferment or enzyme. Since this enzyme does split fat, reduces ethyl butyrate, and shows no tryptic action on fibrin, it is a lipase. In none of the various vertebrates in which it has been injected has it given rise to any evidence of an anaphylactic reaction. Anatomic or histologic lesions were not found in any pancreas of the entire group injected with this purified lipase, which would indicate that this organ had been damaged, and the pancreatic secretion liberated, thus secondarily producing fat necrosis. That is, fat necrosis was produced by the injected lipase, and not through some damage to the pancreas.

As further, and what is considered conclusive, proof that this active substance is a lipase, we have produced lesions of typical fat necrosis in white rats, by injection of a purely synthetic preparation.

TISSUE CHANGES PRODUCED BY THE ENZYME LIPASE

The lipase fraction does show a different reaction on various forms or combinations of body fats and oils. One phase of this is seen regularly as the production of typical fat necrosis in the fat bodies about the testes or uterine horns, the perirenal fat, and the fat in the omentum and mesentery, following intraperitoneal injections of this substance. The anatomic and histologic findings of fat necrosis as produced by the injections of substances herein recorded as having been used in the experimental production of the lesion have been given in detail in a previous publication.[3]

Another manifestation following injections of the lipase fraction from peanuts (no observations have yet been made following injections of lipase from other sources) is that seen in the maturing eggs within the turtle. These eggs in various stages of developmental maturity show definite gross and histologic changes following injections of these substances. Grossly some of the eggs at different periods of development, but more marked in those having a diameter of 3 to 6 mm. appear partially collapsed, and of discus-shape. Within their covering there appears free oil that is almost colorless surrounding the central yellowish pigmented portion. Histologically, these damaged eggs have an intact surrounding membrane. Immediately within this, there is a zone of non-organized, more or less homogeneous, uniformly deep basic staining material, in which appear free globules of fat. The normal eggs in contrast to these, have a central portion of heavy basic staining round bodies surrounded by lighter neutrophilic staining fine granules. The changes in these eggs are attributed to the action of the injected enzyme, lipase. We have not seen this change under any other condition, nor has it been found recorded in the literature. Similar gross lesions have been observed in eggs within gar injected with the same purified lipase.

Foci of fat necrosis to be differentiated from post-mortem changes

Early in this work it was found that in histologic examination of fat tissue from animals dead for several hours before being necropsied, the sections would show a swelling and cloudiness of the cells, with an acidophilic or basophilic homogeneous staining reaction. This post-mortem fatty change must be differentiated from true fat necrosis. Areas of fat necrosis, small or large, are always surrounded by a margin of undamaged fat cells, and when several hours old, by a zone of leukocytic demarcation. These foci do not appear to extend beyond the normal interlobular septa or strands of fibrous tissue. Attention has previously been called by Wells[5] to this limitation by the septa to areas of fat necrosis. The foci of fat necrosis generally show acicular spaces, fat cells swollen, disrupted, cloudy, and the cytoplasm staining acidophilic or basophilic, but the outstanding differential feature is the presence of a leukocytic cellular reaction surrounding the whole area.

CONCLUSIONS

1. Experimental fat necrosis has been produced in the following vertebrates: (A) Cold-blooded: (1) *Lepisosteus platystomus*, (2) *Cyprinus carpio*, (3) *Macroclamys pseudo-geographica*, (4) *Chrysmes belli cinerea*. (B) Warm-blooded: (1) *Gallina domestica*, (2) *Columba livia*, (3) *Mus decumanus*, (4) *Canis familiaris*.

2. Experimental fat necrosis was produced by injections of: (a) pancreatin; (b) solutions prepared from fresh pancreas of the hog (c) pancreatic secretion from dogs, and (d) the purified extracts from: (1) pancreatin, (2) hog's pancreas, and (3) the seeds of Arachis, Helianthus, and Ricinus.

3. The lipase fractions from these animal and vegetable sources contained the active principle for the production of fat necrosis.

4. The active enzyme fulfilled both biological and chemical criteria for a lipase.

5. Fat necrosis was produced in various animals, in which the body temperatures ranged from 18° to 43°C.

6. The data suggest that the production of fat necrosis proceeds more slowly in the cold-blooded vertebrates.

7. There was no species specificity in the action of this enzyme in producing fat necrosis.

8. Post-mortem changes and pancreatic lesions were eliminated as factors in bringing about the findings recorded as fat necrosis.

9. A change in the contained eggs of gravid fish and turtles was observed.

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EDITORIAL

The Prevalence of Intestinal Amebiasis

Intestinal amebiasis is one of the outstanding diseases, the diagnosis of which must of necessity largely depend upon the knowledge of the clinical pathologist.

From the recent past when amebic infection of the human intestine meant amebic or tropical dysentery to the entire medical profession we have come to the present era in the study of this disease when we have ascertained that dysentery is the relatively uncommon acute phase of this infection. Infection of the human being by *Endamoeba histolytica* is of world-wide distribution and of an incidence which appears to be very high in the general population, according to the observations of many students. Just what the general incidence may be is impossible to state at present but newer studies indicate it to be a remarkably common form of parasitism of the human intestine.

In far the majority of instances the infection does not manifest itself as amebic dysentery, and chronic intestinal amebiasis is being given a larger and larger place and more attention by those who are interested in the study of intestinal disease.

We have believed that we know the nature of the disease produced by this ameba. Its tissue invading and destroying qualities have fixed themselves in our conception of a definite pathogenesis and a characteristic pathological picture. If the infection is as widespread and as prevalent as is indicated by accumulating reports, it becomes necessary to determine whether we really do know the whole story of the activities of amebae in the bowel. What constitutes the state of chronic intestinal amebiasis? Is the ameba capable of living its entire life in the lumen of the intestine with effect on the local tissue? If so, is there any systemic effect of such parasitism? Is there a humoral absorption of a "toxic" product? Or does it invariably invade the intestine wall?

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These are problems for the investigation of which many clinical pathologists are favorably situated.

Let it be said at the outset that as a basis for exact and exacting study of amebiasis one should prepare to know the organism. In identifying *Endamoeba histolytica* for the purposes of study in obscure and latent parasitism nothing short of definite recognition of the cyst will probably now suffice. There is too much chance of error by depending entirely upon identification of the trophozoite.

If chronic intestinal amebiasis is as prevalent as commonly indicated and if the ameba is not often simply a lumen dweller, the lesions which characterize it are being overlooked very frequently. The usual post-mortem examination generally carries a rather casual inspection of the intestine, unless it becomes a point of interest from the symptoms presented or because of some readily apparent disease. However, in many places where autopsies are very carefully performed, including a close inspection of the opened intestine, nothing has been discovered to correspond with the conception that amebic intestinal infection is very common. Perhaps the lesions are too small or obscure to be seen even by a careful inspection with the naked eye.

One way in which the problem may be attacked is this: In subjects that have very recently died the large intestine may be tied off in short sections and then each opened and examined separately. The content, particularly that immediately on the mucosa, may be searched by means of fresh wet preparations for amebae. If they are found the section of gut from which they were removed should be studied very thoroughly and minutely, with the hand lens as well as with the unaided eye. Any suspicious spots should be placed in Zenker's fixative, or even if nothing is found, numerous sections of that part should be taken for making histological sections for study.

Such a procedure, as above outlined, might well be instituted with a higher percentage of positive results if routine stool examinations were made on all patients entering large hospitals. Since it is obvious that some of these patients would eventually come to autopsy, it might be expected that a certain number

would be studied sufficiently soon after death so that amebae might be found still alive and hence suitable material for study could be obtained.

It would require a good deal of time to study each case thoroughly, and it would require much care, but it should not be necessary to study any large number of cases before one would have some definite information as to whether *Endamoebia histolytica* may be readily found in the lumen and not in the tissues or that it could be usually demonstrated as a tissue invader in amebiasis. Such a study would have a definite value in the present state of confusion and unsupported hypotheses concerning obscure or latent intestinal amebiasis.

KENNETH M. LYNCH

NEWS AND NOTICES

Tenth Annual Convention of the American Society of Clinical Pathologists

The Committee on Local Arrangements announces the following general program for the meeting to be held in Philadelphia, June 7–9, 1931. The Adelphia Hotel has been selected as convention headquarters. The annual meeting of the Executive Committee of the Society will be held at the Adelphia Hotel on Sunday, June 7. Members of the Executive Committee will receive announcements regarding the place and hour of the meeting. The Scientific Sessions and Scientific Exhibit will be held on Monday, June 8, and Tuesday, June 9, from 9:00 a.m. to 5:00 p.m. at the Philadelphia General Hospital. Programs for the meeting will be mailed in advance to members of the Society. The annual banquet will be held on the evening of June 8 at the Penn Athletic Club. The annual business meeting will be held in the Auditorium of the Philadelphia General Hospital on the evening of June 9.

The tentative program for the Scientific Session is as follows:

Monday Morning, June 8

A discussion of agranulocytic anemia, with report of cases and autopsy findings—H. W. Jones and B. L. Crawford.
The experimental production of agranulocytosis—R. E. Kracke.
Dysplastic granulocytemia—A. M. Weiss and A. A. Goldbloom.
The pathogenesis of acute tuberculous hemoptysis—E. Bogen.
Focal cyclic growth as a factor in the production of nodular goitre—B. Markowitz.

Monday Afternoon, June 8

The starch-iodine reaction in the spinal fluid—B. Gruskin.
The use of the electro-scopometer in routine clinical pathology with new methods—W. G. Exton.
A new modification of the Mallory-Heidenheim differential staining method —J. W. Kernohan.

Experiences in the laboratory diagnosis of tuberculosis with special reference to guinea-pig inoculation—T. B. Magath and W. H. Feldman.

An analysis of 2000 consecutive autopsies—A. O. Brines.

The training of laboratory technicians—R. E. Kracke.

Tuesday Morning, June 9

Active immunization methods against acute, diffuse peritonitis—B. Steinberg.

Experience with bacterial vaccine detoxified with sodium ricinoleate—G. E. Roderick and Ina Maxson.

Standard normals and normal ranges in hematology—F. Boerner.

Physiological properties of the kidney hormone—B. Jablons.

Adenomyoma (endometrial type) of the umbilicus, with report of an additional case—H. Spitz

A case of *Rhinosporidium seeberi* infection—G. S. Graham.

Improved methods in the serum diagnosis of syphilis—H. Engle.

A feature of the program is a symposium on vaccine therapy to be conducted Tuesday afternoon under the auspices of the Committee on Vaccine Therapy. This symposium should be of special interest to members of the Society as well as to general practitioners and it is hoped that there will be a large attendance and that a thorough discussion will result. The following program is announced:

The basic principles of vaccine therapy—John A. Kolmer.

A consideration of the therapeutic and statistical bases for vaccine therapy —N. W. Larkum.

Isolation, selection and preparation of bacteria for vaccines—F. W. Hartman and Edna Jackson.

The use of stock vaccines in respiratory infections in children—Roy P. Forbes.

Bacterial allergy and bacterial densensitization—Warren T. Vaughan.

The therapeutic use of bacteriophage, and its pratical difficulties—Frank B. Lynch, Jr.

Bacterial filtrates in vaccine therapy—John Eiman.

The results of immunization in typhoid fever—S. D. Avery, Major, U. S. A.

Anyone desiring to appear on the program of the Society should communicate with Dr. R. A. Kilduffe before May 15. Those wishing to present exhibits should make requests to Dr. W. S. Thomas, Chairman of the Committee on Exhibits.

Railroad certificates entitling the bearer to half-fare returning

should be secured by members attending the convention. It will be necessary to have these certificates read for the American Medical Association which is in session at the same time, and to have them validated by the proper authorities at the A. M. A. headquarters.

APPROVED LABORATORY TECHNIC

The new book on APPROVED LABORATORY TECHNIC prepared by Dr. John A. Kolmer, Dr. Fred Boerner and Dr. C. Z. Garber with the assistance of a Committee of the American Society of Clinical Pathologists, is now in the hands of the publishers, D. Appleton and Company, with the hope and expectation that it will be published by the time of the meeting in June.

The book is very complete, with adequate illustrations and includes general, clinical pathological, bacteriological, biochemical and serological technic and also a chapter on histological technic by Dr. W. C. MacCarty and Dr. W. L. A. Wellbrock of the Mayo Clinic.

Several years have been spent in its preparation and every method has been revised or passed upon by at least two members of the special Committee of the Society in order to render the technic approved by the A. S. C. P.

An effort has been made to concentrate on single methods for each determination and to give these with sufficient detail for laboratory technicians as well as for assistance to expert pathologists. In many instances, however, alternate methods are included for the purpose of controls or checking wherever it was thought advisable to include them.

The publication of the book is awaited with much interest and with the hope that it will advance the accuracy and status of "clinical pathology" in fulfillment of one of the aims of the A. S. C. P.

RESEARCH COMMITTEE NOTICE

Attention is called to the ruling made last year that two or more abstracts of papers to be considered for the Ward Burdick prize shall be submitted to the committee at least one month

before the annual meeting in order that the committee may properly study the contents before the papers are read.

In regard to the hematological registry it is regretted that only two men have sent in reports of cases of blood dyscrasias, with their slides so far this year. It is earnestly hoped that more will send in their material promptly, so that it can be arranged and studied by the committee long before the next meeting. Please send in blood smears, tissue slides, etc., with all cases. As complete a collection as possible is desired for every case. The conditions particularly included this year are: Acute leukemias, agranulocytic angina, blood dyscrasias following arsphenamines, and cases showing blood pictures resembling pernicious anemia, but due to proved etiology.

Comparative records of urine tests for bile by the Huppert-Nakayama method and other methods should be made for future reporting.

Because of the large expense for animals and labor and other factors, the typing of cultures of tubercle bacilli as outlined will have to be deferred until some later year. Meanwhile it would be good policy to develop technique for culturing tubercle bacilli whenever possible, so that typing later could be done more easily.

A. G. FOORD, *Chairman,*
Pasadena Hospital, Pasadena, Calif.

Openings for clinical pathologists are available in New Jersey and California. Anyone desiring information may write to the Secretary.

BOOK REVIEWS

The Clinical Interpretation of Blood Examinations. By ROBERT
A. KILDUFFE. Pp. xvi + 629, 1931, Philadelphia, Lea &
Febiger, $6.50.

Dr. Kilduffe is known as an able investigator and abstractor of
literature. In this book he has produced a manual that is at once
encyclopedic and very practically useful. The book deals not so
much with the methods of blood examination but, as the title
states, with the clinical interpretations of such tests. The treat-
ment of the various subjects is rather unique and for the student
and laboratory man most valuable in that the author gives ex-
tremely complete summaries of the important work done in the
field with references to the literature.

The outstanding chapters are on blood grouping and on the
Wassermann reaction. The first subject he covers from the
discovery of blood grouping to the most recent work on its relation
to forensic medicine. In the chapter on the Wassermann re-
action he brings out forcefully the pitfalls in the interpretation of
this important test and the fact that all such tests are "laboratory
methods of examination for evidence of reaction to syphilis" and
not tests for syphilis.

Because certain subjects are treated twice in the book some
duplication arises, particularly in the subjects of hydrogen ion
concentration of the blood and sedimentation. The particular
style of subdivision in the book which brought this about may
be open to question.

As is to be expected in the first edition of such a comprehensive
book, certain errors of omission and commission are noticeable.
Thus, polycythemia is omitted from the list of causes of increased
blood volume and sickle cell anemia is said to occur only in ne-
groes, neither of which are, of course, serious errors.

There are splendid discussions of both the cytology and chem-
istry of the blood and the numerous references open to the reader

a vast field of literature in these fields.   The book is an excellent modern summary of knowledge in the field of clinical laboratory interpretation of hematological examinations.

The Factor of Infection in the Rheumatic State. By ALVIN F. COBURN.   Pp. x + 288, 1931, Baltimore, The Williams & Wilkins Company, $6.00.

The author states in the introduction that "the purpose of this study is to give a comprehensive description of the rheumatic state with its many phases."   This he has ably put forth in a series of case histories from which he has drawn certain conclusions.   To arrive at the picture of this mosaic disease he has examined more than 3,000 rheumatic subjects, more particularly 162 whom he was able to observe over a long period of time. From these studies he has shown that Cheadle's conception of rheumatic fever is essentially correct and that the rheumatic state is distinguishable from other forms of illness, although protean in its manifestations.   In order of frequency the most common of these are: Polyarthritis, pancarditis, epixtaxis, muscle pains, pallor, headache occurring with the attacks, cardiac pain, chorea and abdominal symptoms, while skin manifestations and subcutaneous nodules are fairly common.

The author emphasizes the importance of upper respiratory and pulmonary infections as forerunners of the disease.   The effect of these and other environmental influences was studied in four widely separated groups of rheumatic people including one colony in Porto Rico.   From the study of the New York Hospital group he concludes that there is (1) probably some annual variation, (2) seasonal incidence — the majority of cases appear in the early spring, (3) geographic distribution — almost unknown in the tropics, (4) familial tendency, and (5) influence of the environment of the city.   This was studied particularly in immigrants in whom the disease developed for the first time after settling in New York.   However, he states that exposure to upper respiratory diseases is more important than other environmental factors.

A large share of the book is devoted to bacterial studies on rheumatic patients and controls.   From a large series of carefully

performed blood cultures he concludes, in opposition to Cecil, that bacteremia is not a part of the entity. He brings forth evidence to show the importance of upper respiratory infections as the genesis of the rheumatic state and believes that the important factor is the absorption of toxins from hemolytic streptococci found so frequently in the throats and tonsils of such persons. He definitely recognizes, however, that the physiological state of the individual also plays an important part. As a part of his proof for such a causal relationship to streptococci he details experiments with nucleoproteins obtained from bacteria from the throat and injected into the skin, from which he obtained fairly typical reactions.

It is unfortunate that he fails to recognize that "Streptococcus hemolyticus" really means nothing bacteriologically speaking. No such name appears in Bergey's Manual; many different streptococci produce hemolysis. If he means to incriminate any hemolytic streptococcus he should not use a Latin binomial. Apparently he does not so mean to use the term, yet he does not furnish evidence to support the view that all the organisms he isolated as hemolytic streptococci belong to one species. There is great need to study further the organisms with which he deals.

The book is printed in the usual faultless style of Williams & Wilkins, is well illustrated, and contains many tables and colored plates, one of which illustrating the anaerobic nature of the organism isolated in one case could well have been omitted.

The book will not only take its place on the shelves of serious students of the laboratory side of medicine, but will be useful and appropriate on the desk of the busy practitioner.

The Rôle of the Streptococci in Scarlet Fever. By DAVID THOMSON and ROBERT THOMSON. Annals of the Pickett-Thomson Research Laboratory, Vol. 6, pp. xiii + 470, London, Bailliere, Tindall and Cox, and Baltimore, Williams & Wilkins Company.

This is an extensive and valuable collection of the literature which includes 1,400 papers. The authors point out in the preface that this literature represents 1,000 years of work. They conclude that "the weight of evidence shows scarlet fever to be

due to a specific streptococcus, the *Streptococcus scarlatinae*."
In the summary are listed fifteen types of experimental evidence
to support this contention. Such evidence is based upon (1)
cultural differences, (2) fermentation tests, (3) agglutination
tests, (4) opsonic tests, (5) precipitin tests, (6) specificity of toxin,
(7) production of rash by toxin, (8) production of rash by vaccine,
(9) Schultz-Charlton phenomenon, (10) curative action of anti-
serum, (11) production of short immunity by antiserum, (12)
immunization by toxin and vaccine, (13) experimental production
of scarlet fever, (14) milk borne epidemics, and (15) contraction
of scarlet fever in the laboratory.

A more conservative opinion after examination of the literature
on these various points would be that scarlet fever is due to a
hemolytic streptococcus, but proof as to its specificity is far from
complete. If, for example, one were to examine the results of
fermentation tests cited by the authors to differentiate the strep-
tococcus of scarlet fever from other types of hemolytic strepto-
cocci, one would find that of fourteen reactions eight are variable.
While it may be true that the streptococci from scarlet fever will
give a certain set of reactions more frequently than streptococci
from other diseases, yet the notable exceptions to be found in the
fermentation tests, and in most of the other tests set up as criteria
of specificity, remain to disturb the critically-minded person and
serve as a commentary on the difficulty of the problem.

<div align="right">LUTHER THOMPSON.</div>

PATHOLOGICAL ANATOMY AS THE KEYSTONE OF THE PRACTICE OF CLINICAL PATHOLOGY*

KENNETH M. LYNCH

Charleston, South Carolina

The ultimate success of a professional specialty is contingent upon the attainment of "eminence in the performance of function." This I take to be the primary interest of the members of this organization.

The speciality represented here is a product of the heaping of tests and procedures to be used in diagnosis upon a profession largely untrained to make use of them. If, or when, the time may come that other practitioners may be competent, either by themselves or by means of purely technical assistance, to perform the laboratory procedures applicable to their own practice there will be no further need of the specialty.

In an estimate of the field of medical practice encompassed by clinical pathology there seemed to be three reasons for the need of recognized specialty at the present time. The first is that a large part of the practitioners of the present are untrained in the performance of methods of laboratory diagnosis; the second, that most if not all are unable to correctly interpret the applicability and the results of such tests; and the third is that a part of such laboratory diagnosis may be of such a nature as to demand such special training and experience as to create within itself a legitimate specialty.

The first of these reasons results from the fact that laboratory diagnosis has grown into medicine in mushroom fashion within a comparatively few recent years. Consequently probably the majority of physicians still in active practice were never schooled

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* Presidential Address, read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

in most of the methods now subject to every day general use. Graduates in medicine of the present time, however, are well schooled in laboratory medicine in general and as internes usually obtain sufficient experience to be reasonably competent in most of the methods which they will use in practice. Is it likely, and is it economically sound that they should abandon the benefits of this training when they enter practice? Is it necessary that they should turn it over to a specialist?

If any practitioner does his own laboratory work as well as a clinical pathologist, or if it is as practically serviceable, there is no reason why he should not and it will be economically and generally to his advantage to do so. Consequently we may expect to see and, I believe, are now seeing a tendency in the younger generation of laboratory trained physicians to make their own laboratory examinations. As their practice becomes more pressing so that they may profitably do so they may and will, no doubt, obtain the services of technical assistants, commonly in association with their colleagues.

The clinical pathologist makes much of the second reason as a justification for the specialty, that is the inability of other practitioners to interpret and make full use of laboratory examinations. This is also because a large part of the profession is unschooled in laboratory medicine. This condition, like the first, may not be one of long life. In fact it is less likely to hold with the newer generation than is that of incompetency in the actual performance of laboratory measures. The newer generation is well schooled, and will continue to be, in the interpretation of the usefulness and meaning of the various tests and procedures available. Sanford[1] thinks that the clinical pathologist will be the connecting link between the pure research laboratory and the application of its discoveries and inventions in the practice of medicine, that he will keep before the other practitioners the newer advances and interpret them in terms of actual usage. Perhaps so, but one may be skeptical that modern internists are going to be far behind clinical pathologists in knowledge of and

[1] SANFORD, A. H. The rôle of the clinical pathologist. Jour. Am. Med. Assn., 95: 1465–1467. 1930.

practical interpretation of the available measures and methods which come from the medical research mill.

If other practitioners are going to wait supinely to be so informed there will be need of such a go-between, but a specialty built upon the shortcomings of others can live only so long as those deficiencies are not corrected.

It seems probable that other practitioners will be apt to undertake less the actual laboratory labor than the interpretation of its usefulness and meaning. Well educated physicians can just as well interpret laboratory examinations as clinical pathologists, it is conceivable that they may even believe that their own interpretations would be better in their own clientele. They can, also, do or cause to have done by supervised technicians the actual examinations in the clinical laboratory investigations now in use or apparently likely to be used. On these two counts, therefore, and if this constitutes the whole case of laboratory medicine, the permanency of clinical pathology as a definite medical specialty may be seriously questioned.

This makes the third reason the one of primary consideration. Is there a part or are there things in the actual doing of laboratory examinations which within themselves necessitate a complete medical training and expertness beyond that to be expected of other practitioners?

It needs only that one make a scanning review of the content of a text or manual on clinical laboratory examinations to be convinced that there is nothing necessarily included which may not be done by a properly and adequately trained person without any general knowledge of medicine. In so far as the performance of the actual tests and procedures and the setting down of the results goes it needs only that the doer shall be competent in an honest and reliable technique. As a matter of fact beyond the obtaining of some of the materials to be examined and the actual interpretation of the results in terms of diagnosis the strict field of clinical pathology as usually set down may not constitute the practice of medicine. In so far as an individual may make an examination for tubercle bacilli in the sputum, for albumin in the urine, or for an organism in the stool, these acts and their results

are complete within themselves. They require no general medical training and so long as the examiner assumes no responsibility in the withdrawing of materials from or other handling of the person of a patient or in the making of a dignosis to and for the patient, and so long as he or she does not enter into the treatment of the case, these things are not the practice of medicine and the laboratory examiner is responsible to no one in the matter except the physician in whose charge the patient remains.

If, however, any act concerned in or related to the performance of laboratory examinations constitutes the practice of medicine, the performer must be subject to the same considerations as other physicians in the matter of education and licensure.

In the practice of clinical pathology as now done there are numerous acts which do constitute the practice of medicine and for which the examiner is directly responsible to the patient and the state. The withdrawing of blood, body fluids, secretions, excretions, etc.; the injection or other administration of materials for functional studies; the taking of material for bacteriological studies; the performance of metabolic determinations; the injection of vaccines; the application of allergic tests; these and other quasi-clinical measures in diagnosis and in therapy come within the realm of medical practice and make it necessary that the medical laboratorian who does them shall be a licensed physician.

So long as the work comes within the field of practice it constitutes a medical specialty but it may be that it is so constituted now as a matter of practical convenience to the profession on account of conditions which may be expected to change.

These features of actual practice of medicine may, perhaps, be done by the clinician in the case, along with the interpretation of the results of such examinations, thus retaining the whole medical situation in his hands and leaving the medical laboratorian as a detached technician.

Individually considered it would seem that there is none of the practices in medical laboratories, as done at the present, which may not be handled completely by the clinician. If this is the case they should be handled by him, in considerations of efficiency and economy.

While this seems to be logical when these practices are considered individually or piece-meal it is their collective volume which makes it difficult for clinicians to encompass even the field which may be concerned in their own personal business and which makes of clinical pathology to no inconsiderable extent a specialty not of necessity but of convenience.

The gathering together of all the facilities and qualified personnel for a complete medical laboratory is generally beyond the opportunity of individual physicians, but may readily be accomplished by physicians acting in concert. As a matter of fact we now see this occurring in coöperative groups of practitioners of many varieties.

The increase of hospitalization of sick people has an important bearing here. In hospitals the laboratory facilities for a community or a certain clientele may be concentrated and it is an increasing custom to send patients into hospitals for diagnostic study on account of the availability of such facilities there. In hospital laboratories certain classifying agencies now demand that the work be under the direction of specialists of certain qualifications. In private medical laboratories which are recognized by these agencies there is a like requirement. In fact this specification of a special training in addition to a general medical education is the main pillar upon which the structure of the specialty now stands.

There is one part of the practice of pathology as a whole which probably clinical pathologists might well take into more serious consideration in guiding the future of this as an enduring medical specialty.

We have had for the duration of modern medicine the specialist known as the pathologist. Until recent times he has been mainly a teacher of general pathology in medical schools. In the practice of medicine of the present he has had an offshoot known as the surgical pathologist, whose business it is to make diagnoses from the study of diseased tissue and whose particular usefulness lies in the diagnosis of tumors, the differentiation of tumors from other pathological states, and the interpretation of the qualities of tumors by their characteristics. Along with such pathological diagnostic and prognostic studies and the routine post-operative

judgment of surgically removed tissues of all kinds, it has come into prominent recognition that the development of autopsy services as a check upon and a guide to clinical work and as a postgraduate educational measure for hospital staffs, is a major function of the hospital pathologist.

Now no one may question the necessity for a thorough general medical education and an extensive well tutored experience in this work in the qualification of a pathologist worthy of the name. There can be no question that here is constituted a medical specialty as exacting in its demands of expert medical knowledge as any other division of medicine; and it is probably a correct interpretation that the unquestionable necessities in this case rather than of those concerned with so-called clinical laboratory work are responsible for the rigid requirements set down for the hospital pathologist and the practicing pathologist in the private laboratory.

There exists at the present some distinction between a pathologist and a clinical pathologist, in that the former professes to be competent in general pathology and tissue diagnosis while the latter carries in common conception mainly proficiency in clinical laboratory work.

While in medical schools and large hospitals, particularly teaching centers, with the degree of specialization within specialties necessary to the organization of such institutions, there will, no doubt, continue to be specialists in general pathology, surgical pathology (and even subdivisions of that at times), and in clinical laboratory diagnosis, in general practice it would seem that logically the practicing pathologist should cover all of these branches.

Particularly would this seem to be the case in pathology services of hospitals, and that appears to be the purpose of the profession. It is doubtful that, even in the extent of specialization which is the fashion of the day and appears to be the trend toward the future, there is need or will be room for separation of this field into pathology and clinical pathology. It is for the purpose of arguing this matter, as of vital consideration in looking into the future of the interests of this membership, that these

questions are here presented and their evaluation attempted. It is of foremost importance that we shall analyze our own qualifications and merits, that we shall guide our own destiny to the credit of our specialty and not blindly pursue a path of present opportunity and convenience to the eventual dissatisfaction of ourselves and of the profession who now trust to us the development of the field of practical laboratory medicine.

To properly prepare oneself to practise pathology as a whole is an accomplishment of no mean proportions and of no little consumption of time. There may be those who will deny me the right to say that preparation for competency in tissue diagnosis and in judgment of morbid anatomy so far exceeds that for clinical laboratory diagnosis that the latter may be relegated to a comparatively unimportant second. That is a deliberated judgment resulting from twenty years experience, including the teaching of general pathology, the direction of a teaching hospital division of pathology, and the private practice of clinical pathology. I would go so far as to estimate that six years in the former is productive of less competency than six months in the latter. Perhaps that is the reason for the separation of pathology and clinical pathology, as conceived at the present time by the profession at large.

This is no disparagment of the qualifications of the membership of this Society of high ideals and objectives. It is our purpose to so guide the practice of pathology that it shall occupy its rightful place in the profession. In order that it may do so let us not be misguided into the line of least resistance for immediate purposes but let us logically take into account the conditions as they are now and as they will reasonably develop. Let us recognize that here we have a specialty subject to absolute control within the profession itself and, as long as it does not encroach actually upon clinical medicine, free of the commercial advantages which clinical medicine has. It does not involve pleasing the uninformed patient, it only involves satisfying the informed profession. In satisfying the profession now and in the future the practicing pathologist will find that proficiency in the science of morbid anatomy is the keystone of the structure of his specialty and that with this as the foundation the wide field of clinical laboratory

diagnosis will no doubt be left in his hands. The clinical pathologist may probably come to mean the practicing pathologist, mainly as a hospital functionary, as distinguished from one who teaches pathology.

A COMPARISON OF DIFFERENT CULTURE METHODS FOR THE ISOLATION AND GROWTH OF MYCO-BACTERIUM TUBERCULOSIS

AN EXPERIMENTAL STUDY

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Petroff, in 1915, demonstrated the practicability of isolating and growing *Mycobacterium tuberculosis* directly from infective material, and considerable impetus has been given this phase of research in tuberculosis by the use of methods described by Corper, Corper and Uyei,[3] Sweany and Evanoff, Miraglia, and others. Since difficulties were seldom experienced in obtaining cultures of the avian form of *Mycobacterium tuberculosis* on any of the mediums described by these authors, and since the suitability of each of these cultures for the production of growth of the human and bovine forms of the organism seemed to vary within wide limits, an experiment was planned to determine, if possible, which of the mediums used was the most dependable for the demonstration of acid-fast bacilli from spontaneously infected human and bovine material.

The use of suspensions of *Mycobacterium tuberculosis* prepared from cultures was purposely avoided since the bacteria in such suspensions cannot be considered comparable in cultural demands with organisms whose growing propensities have not been enhanced by previous residence on artificial mediums. One objection to the use of spontaneously infected material in a study of this kind is the difficulty of securing comparable numbers of the organisms in each culture tube inoculated. However, if many bacteria are present and several tubes of each kind of medium are inoculated it should be possible to obtain a fairly definite idea as to the ability of the respective mediums to promote the growth of *Mycobacterium tuberculosis*.

The tuberculous character of most of the specimens secured for this study was presumed by the presence in the infective material of acid-fast bacteria morphologically indistinguishable from *Mycobacterium tuberculosis*. In specimens in which acid-fast bacteria were present on the microscopic smears the organisms varied in number from few to many. The number of organisms observed varied with the character of the material examined, being most numerous in smears prepared from sputum and less abundant in smears prepared from the bovine material. Although the sputum and human tissues and the bovine material were examined for acid-fast organisms by the preparation of direct smears the urine was examined by preparing smears from the precipitate resulting from centrifuging 15 cc. of the original specimen.

MATERIAL USED

The material of human origin was obtained from the Section on Clinical Pathology and the Section on Surgical Pathology of The Mayo Clinic and consisted of the following: thirteen specimens of urine voided and catheterized from the bladder, ten specimens of sputum, and four specimens of tissue. The specimens of sputum and urine were received in the containers in which they were obtained from the respective patients, and the infective tissues were received in sterile vials or test tubes. By appropriate staining methods acid-fast organisms morphologically similar to *Mycobacterium tuberculosis* were observed in all but one of the twenty-seven specimens. This specimen (case 7, table 1) was obtained from an infected bursa of the elbow joint and will be considered in detail later in this report.

The bovine material was obtained through the courtesy of the Federal Meat Inspection authorities from various abattoirs in South St. Paul, Minnesota. Nineteen separate specimens representing as many different animals were obtained. The specimens which were forwarded to the laboratory packed in dry borax consisted of the following: seven bronchial lymph nodes; six mediastinal lymph nodes; one cervical lymph node; one mesenteric lymph node; one specimen of lung tissue; one of a subcutaneous lesion, and two specimens of lymph tissue of undetermined origin. Smears from all but five of the bovine lesions were positive for acid-fast bacilli.

METHOD OF INVESTIGATION

If the material consisted of tissue it was emulsified by grinding in a sterile mortar in sterile physiologic sodium chloride solution. The fluid portion was

transferred to a test tube and the heavier particles were permitted to settle. This required from one to several hours. Material containing much mucus such as tenacious sputum was washed in a small amount of physiologic sodium chloride solution by vigorous shaking, and the heavier particles were permitted to settle as for emulsified tissues. The respective specimens of urine were used without preliminary concentration by centrifugation.

Two guinea pigs were each inoculated with 1 cc. portions of the same material used for cultural attempts. One of the guinea pigs was inoculated subcutaneously and the other intraperitoneally. Each pair was placed in a separate cage and those living at the expiration of eight weeks were killed for necropsy. The presence or absence of a tuberculous infection was determined by the recognition of characteristic gross lesions. In doubtful cases histologic sections were prepared and stained for the presence of acid-fast organisms by a method previously described.[11]

Two 1 cc. portions of each specimen were each treated with 5 per cent oxalic acid solution, as recommended by Corper and Uyei,[4] and similar amounts were treated with 3 per cent sodium hydroxide, as suggested by Petroff, and by Sweany and Evanoff. This was done for the purpose of killing or inactivating contaminants which may have been present. After centrifugation all but approximately 0.5 cc. of the supernatant fluid was discarded and with a capillary pipet the precipitate was thoroughly mixed with the remaining fluid. The suspensions treated by oxalic acid were mixed with each other as were the suspensions which had been treated with sodium hydroxide and the respective suspensions were then distributed on the surface of the various culture mediums with the aid of the capillary pipet.

From each specimen, cultures were attempted on the following mediums: crystal-violet-glycerine-water-potato medium of Corper and Uyei;[3] veal-egg-cream-milk medium of Sweany and Evanoff which was slightly modified,* and

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* In order to overcome the difficulty of obtaining a sufficient amount of filtrate from the meat-milk mixture as described in the original method, the following modification was followed: 250 grams of freshly ground round steak was added to 100 cc. of distilled water and soaked overnight at ice box temperature. It was then autoclaved at 15 pounds for fifteen minutes. While yet warm and without filtering, a mixture as follows was made: autoclaved meat broth, 100 cc.; sterile standard cream, 50 cc.; sterile whole milk, 50 cc.; eggs (whites and yolks), twice the total of the meat-broth-cream-milk mixture, or 400 cc. After being thoroughly stirred and strained through four layers of sterile gauze the medium was tubed and sterilized according to the procedure given in the original method except that the sterilization process was continued for four days instead of three.

the glycerine-broth-egg yolk medium of Miraglia.* From each of the specimens of human origin six tubes of each kind of medium were inoculated with material treated with oxalic acid, and a similar number were inoculated with the material which was treated with sodium hydroxide. With the material of bovine origin eight tubes of each kind of medium were inoculated with the material which had been treated with oxalic acid, and eight tubes were inoculated with material treated with the sodium hydroxide. The tubes were incubated at 37°C. and were examined after the lapse of one week and twice weekly thereafter.

RESULTS

A summary of the more important results obtained from the work with the twenty-seven specimens obtained from human sources is recorded in table 1.

From the foregoing data it is evident that a positive diagnosis of tuberculous infection was made in 24 or 88.8 per cent of the cases by the presence of lesions of tuberculosis in the inoculated guinea pig. By cultural means acid-fast organisms morphologically similar to *Mycobacterium tuberculosis* were present in twenty-three (85 per cent) of the cases. Each of the three cases which were negative by guinea pig inoculation were also negative by the cultural procedures although the culture tubes were observed for sixty-seven, eighty-seven, and seventy-two days, respectively, before being discarded. On the other hand, material from one of the cases in which acid-fast bacilli were not isolated by cultural means induced unmistakable lesions of tuberculosis in one of the inoculated guinea pigs. The material in this case (case 7, table 1)

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* The medium of Miraglia was prepared as follows: seven fresh eggs were washed and immersed for ten minutes in 80 per cent alcohol; the yolks only of the seven eggs were added to 100 cc. of 6 per cent glycerine broth (the original method calls for 5 per cent glycerine); the ingredients were thoroughly mixed with an egg beater and tubed; the mixture was sterilized in the Arnold sterilizer or the insipissator, first day at 75°C. until solidified, then 85°C. for one hour, and on second, third and fourth days for 75°C. for one hour; it was incubated for two days at 37°C. before using. The glycerine broth was made as follows: Meat extract, 3 grams; peptone, 10 grams; glycerine, 48 cc., and distilled water sufficient to make 1,000 cc. were mixed. The reaction should not be alkaline and it is usually sufficiently acid without further adjustment. The broth was boiled for fifteen minutes, cooled, and filtered through paper. Distilled water was then added to restore the original volume and the broth was sterilized in the autoclave for fifteen minutes at 15 pounds pressure.

consisted of a portion of the infected bursa of the elbow joint of an adult human being. A direct smear prepared from the diseased tissue failed to disclose acid-fast organisms. Since it was thought possible that the infection might represent a bovine

TABLE 1

Character of Infectious Material of Human Origin and Results of Cultural Attempts to Isolate and Grow Acid-fast Bacilli

NUMBER	MATERIAL	DIRECT SMEAR	RESULT OF GUINEA-PIG INOCULATION	CULTURE TUBE	RESULTS ON RESPECTIVE MEDIUMS		
					Corper and Uyei	Modified Sweeny-Evanoff	Miralgia
					days	days	days
1	Kidney	+	+	+	+ 25	+ 25 C	+ 25
2	Urine	+	+	+	15 C	+ 64	15 C
3	Urine	+	+	+	+ 35	+ 49 C	+ 19 C
4	Urine	+	+	+	+ 28	+ 77 C	+ 14
5	Urine	+	+	+	+ 28	+ 35	+ 18
6	Urine	+	+	+	− 60	+ 36 C	Not used
7	Bursa	−	+	−	− 88	Not used	− 88
8	Kidney	+	+	+	+ 13	+ 13	+ 13
9	Sputum	+	+	+	+ 21	+ 24	+ 17
10	Sputum	+	+	+	+ 34	+ 27	+ 16
11	Sputum	+	+	+	+ 21	+ 21	+ 14
12	Urine	+	−	−	− 67	− 67	− 67
13	Sputum	+	+	+	− 72	+ 31	+ 21
14	Sputum	+	+	+	+ 30	+ 20	+ 34
15	Sputum	+	+	+	+ 17	+ 17	+ 10
16	Sputum	+	+	+	+ 22	+ 26	+ 15
17	Sputum	+	+	+	+ 20	+ 16	+ 16
18	Sputum	+	+	+	+ 27	+ 20	+ 16
19	Sputum	+	+	+	+ 26	+ 19	+ 15
20	Urine	+	Failure*	−	− 87	− 87	− 87
21	Urine	+	+	+	+ 59	+ 59	+ 46
22	Urine	+	+	+	+ 30	+ 30	+ 45
23	Kidney	+	+	+	+ 24	+ 13	+ 24
24	Urine	+	+	+	+ 32	11 C	11 C
25	Urine	+	+	+	13 C	+ 56	13 C
26	Urine	+	−	−	− 72	− 72	− 72
27	Urine	+	+	+	− 65	+ 45	+ 31

* Died within three weeks after injection. Lesions of tuberculosis were not present.

C = Contaminants present in all tubes receiving the inoculum treated by oxalic acid and those receiving the material treated by sodium hydroxide.

Note: Bold-face type shows instances in which growth first appeared.

form of tuberculous infection, rabbits were inoculated intravenously with portions of the same material used in the guinea pig inoculations and the cultural attempts. The rabbits were without discernible lesions of tuberculosis after a lapse of sixty days. The presence of lesions in one of the inoculated guinea pigs seemed sufficient to consider the infection in this instance as due to the human type of *Mycobacterium tuberculosis*. There were three cases in which data were negative by both animal inoculation and cultural means. In only one case was there failure as a consequence of the guinea pig inoculations due to the early death of both of the inoculated animals (case 20, table 1). In very few instances was there sufficient contamination on the culture mediums to attribute to this cause the subsequent failure to obtain colonies of acid-fast bacilli. It is true that not infrequently contaminating bacteria occupied the same medium as colonies of Mycobacterium, but the latter usually were sufficiently characteristic to make their recognition easy. Often the contaminating bacteria did not become visible until the colonies of acid-fast bacilli were well established.

Comparing the efficiency of the respective culture mediums used to promote the growth of acid-fast bacilli from the material of human origin, it is apparent that the medium of Miraglia proved superior to the others in promoting the early growth of these microörganisms. In thirteen of the cases growth appeared first on the medium of Miraglia whereas in five cases growth was first observed on the modified medium of Sweany and Evanoff. Material from one specimen yielded colonies of acid-fast bacilli which appeared simultaneously on the medium of Miraglia and the medium of Sweany and Evanoff modified as described. Colonies first appeared on the medium of Corper and Uyei in only one instance (case 24, table 1) although in two of the specimens (cases 1 and 8, table 1), growth appeared on all three of the mediums simultaneously and in one instance colonies were first observed on the same date on both the crystal-violet-glycerine-water-potato medium of Corper and Uyei and on the modified medium of Sweany and Evanoff (case 22, table 1).

With the medium of Corper and Uyei acid-fast cultures were

not obtained from four of the specimens which were proved to be tuberculous by the inoculation of guinea pigs. In two other instances the medium of Corper and Uyei was so badly overgrown with contaminants after thirteen and fifteen days, respectively, that any subsequent growth of acid-fast bacilli was precluded.

On the modified medium of Sweany and Evanoff there was but one failure to obtain a growth of acid-fast bacilli from portions of the same material which produced lesions of tuberculosis in guinea pigs and in this instance the surface of all the culture mediums was completely overgrown with contaminating microorganisms after eleven days of incubation.

With the exception of three failures due to the growth of contaminating bacteria the medium of Miraglia failed to yield colonies of acid-fast organisms in only one instance in which the tuberculous character of the inoculum was proved by lesions in an inoculated guinea pig.

Although the earliest colonies occurred in most instances on the medium of Miraglia the largest number of specimens found to contain acid-fast bacilli was revealed by the modified medium of Sweany and Evanoff. Of the twenty-four specimens which were finally adjudged tuberculous as a consequence of guinea-pig inoculations twenty-two yielded acid-fast bacilli on the modified medium of Sweany and Evanoff, whereas the medium of Miraglia promoted growth from nineteen and that of Corper and Uyei's potato medium from eighteen.

In those tubes in which a growth of acid-fast bacilli occurred the growth on the modified medium of Sweany and Evanoff was perhaps the least luxuriant. Generally speaking, the bacterial growth was most abundant on the medium of Miraglia although growths of comparable luxuriance resulted in many instances on the medium of Corper and Uyei.

Most of the colonies which were examined microscopically consisted of pure cultures of acid-fast bacillary forms although in a few instances spores and yeast-like forms were observed in addition to the acid-fast bacilli. The pathogenicity of the acid-fast cultures isolated from the respective specimens obtained

from human sources was not demonstrated by animal inoculations. Therefore the designation of these organisms as *Mycobacterium tuberculosis* is entirely presumptive. More work on this phase of the problem is contemplated.

TABLE 2

CHARACTER OF INFECTIOUS MATERIAL OF BOVINE ORIGIN AND RESULTS OF
CULTURAL ATTEMPTS TO ISOLATE AND GROW ACID-FAST BACILLI

NUMBER	MATERIAL	DIRECT SMEAR	RESULT OF GUINEA-PIG INOCULATION	CULTURE	RESULTS ON RESPECTIVE MEDIUMS		
					Corper and Uyei	Modified Sweany-Evanoff	Miraglia
					days	*days*	*days*
1	Bronchial lymph node	+	+	+	+ 31	+ 27	+ 50
2	Mediastinal lymph node	+	+	+	+ 78	+ 26	+ 42
3	Bronchial lymph node	+	+	+	− 119	+ 14	− 119
4	Bronchial lymph node	+	+	+	+ 14	+ 14	+ 14
5	Lung	−	+	+	+ 84	+ 26	+ 41
6	Mediastinal lymph node	+	+	+	− 87	+ 21	+ 21
7	Subcutaneous lesion	−	−	−	− 110	− 110	− 110
8	Lymph tissue	−	+	−	− 105	− 105	− 105
9	Lymph tissue	+	+	+	+ 49	+ 43	− 105
10	Mediastinal lymph node	+	+	+	+ 85	+ 36	− 90
11	Mediastinal lymph node	−	+	+	− 90	+ 76	− 90
12	Mediastinal lymph node	+	+	+	− 95	+ 48	+ 82
13	Bronchial lymph node	+	+	+	+ 50	+ 44	− 71
14	Mesenteric lymph node	−	+	+	+ 85	+ 24	− 85
15	Cervical lymph node	+	+	+	+ 85	+ 51	− 85
16	Mediastinal lymph node	+	+	+	+ 84	+ 30	− 84
17	Bronchial lymph node	+	+	Failure*	21 C†	21 C*	21 C*
18	Bronchial lymph node	+	+	+	− 83	+ 49	− 83
19	Bronchial lymph node	+	+	+	+ 76	+ 36	+ 57

* The culture became overgrown with contaminating bacteria before the elapse of sufficient time for possible colonies of Mycobacterium to appear.

† C = Contaminants present in all tubes receiving the inoculum treated by oxalic acid and those receiving the material treated by sodium hydroxide.

Note: Bold-face type shows instances in which growth first appeared.

In the series of nineteen specimens (table 2) obtained from bovine sources a definite diagnosis of tuberculosis was made as a consequence of guinea-pig inoculation in eighteen, approximately

95 per cent. By cultural means colonies of acid-fast bacilli were obtained from sixteen of the nineteen specimens, approximately 84 per cent. The bovine specimen (case 7, table 2) which was negative following the inoculation of guinea pigs remained negative by cultural means although the observations were continued for one hundred ten days before the culture tubes were discarded. The failure to demonstrate the presence of a tuberculous infection in this specimen should not detract from the reliability of either of the procedures used since this case belonged to a group of subcutaneous infections known as "skin lesions" which are not infrequently found in cattle which react to tuberculin. The attempt to incite lesions of tuberculosis in inoculated guinea pigs or to demonstrate the presence of *Mycobacterium tuberculosis* by cultural procedure has usually been unsuccessful and consequently the tuberculous character of lesions similar to this has not been established.

In the bovine specimens from which acid-fast bacilli were eventually isolated, the colonies appeared first on the modified medium of Sweany and Evanoff in every instance except two. In one instance (case 4, table 2) growth appeared on all mediums simultaneously, whereas in the other, colonies appeared at the same time on the modified medium of Sweany and Evanoff and the medium of Miraglia (case 6, table 2). In eleven of the nineteen attempts at growth, acid-fast bacilli were observed in the potato medium of Corper and Uyei.

In case 5 (table 2) a tube of potato medium which had been inoculated with material previously treated with 3 per cent sodium hydroxide solution developed after eighteen days a yellowish, somewhat viscid colony approximately 0.2 cm. in diameter. This colony was found to consist of acid-fast bacilli which morphologically were significantly like *Mycobacterium tuberculosis*. Pathogenicity could not be demonstrated, however, for guinea pigs, rabbits, or chickens. The original colony eventually lost its yellow color and became a dirty gray, and attempts to obtain transfers on fresh mediums failed.

Miraglia's medium promoted the growth of acid-fast bacteria in only seven of the nineteen bovine cases. Two of the seven

positive results which were obtained with Miraglia's medium represented different cases than those which were positive with the medium of Corper and Uyei.

In only one instance did the bovine specimens yield an overgrowth of contaminating bacteria which might be considered as having occurred too soon to permit the eventual growth of acid-fast bacilli (case 17, table 2). The failure of any of the mediums to promote growth of acid-fast bacteria (case 8, table 2) while the tuberculous character of the material was proved by the development of lesions following guinea pig inoculations constitutes evidence to substantiate the contention that the inoculation of guinea pigs is a more reliable procedure in the demonstration of certain tuberculous infections than cultural means.

In the experiments with the bovine material the tubes which were finally adjudged as negative were kept under observation for at least twelve weeks and a few were retained for as long as one hundred five to one hundred nineteen days before being discarded.

As in the case of the cultures obtained from the material of human source, animals were not inoculated with each of the acid-fast strains isolated from the bovine material and consequently their capacity to induce tuberculosis in susceptible animals was not proved. Further work on this phase of the problem is in progress.

The early appearance of colonies of acid-fast bacilli on all mediums in case 4 incited some doubt as to the true character of the organisms. Tests of pathogenicity subsequently proved the organism to be *Mycobacterium tuberculosis* of bovine origin since it was found to be markedly pathogenic for guinea pigs and rabbits but not for chickens.

A comparison of the results from the use of 5 per cent oxalic acid and 3 per cent sodium hydroxide for the control of contaminants failed to reveal any outstanding or consistent difference. In most of the specimens each of the reagents proved satisfactory in the elimination of nonacid-fast bacilli. It was also observed that occasionally material was encountered in which neither of these was successful in preventing the development of contami-

nants. Apparently if the contaminating organisms are excessive or if of a type which is particularly resistant to oxalic acid and sodium hydroxide in the concentrations used, neither of the reagents will effectively eliminate them from subsequent cultures.

As to the possible deterrent effect of these reagents on *Mycobacterium tuberculosis* these data fail to disclose anything of significance. In one instance (a human specimen, case 8, table 1) all mediums which were seeded with the material which had been treated with sodium hydroxide yielded acid-fast colonies consistently earlier by one week than the mediums seeded with the material which had been treated with the 5 per cent oxalic acid. In two other instances, however (cases 16 and 19, table 1), the tubes which were inoculated with the material previously exposed to 5 per cent oxalic acid developed acid-fast colonies before those which had received the material treated with 3 per cent sodium hydroxide. Another interesting observation occurred in case 21 (table 1) in which positive results were obtained from all mediums except the crystal-violet-glycerin-water-potato tubes which had been seeded with material treated by sodium hydroxide. Since the growths were luxuriant on all the other mediums it is hardly likely that the failure of a growth to appear in the potato tubes in which was material that had been exposed to the sodium hydroxide solution, was due to the absence of acid-fast bacilli in the particular inoculum used. It is evident that although certain minor differences may be noted in a comparative study of these two reagents, the use of either one would give satisfactory results in most of the specimens from which cultures may be attempted. If the material to be cultured is plentiful, the possibility of obtaining positive results in certain instances would probably be enhanced if both reagents were used for the elimination of contaminating bacteria.

As to the germicidal value of crystal violet, in the potato medium of Corper and Uyei there was no evidence that the presence of this dye had any appreciable effect on the development of bacteria other than *Mycobacterium tuberculosis*. It is true that few of the potato tubes developed growths of contaminating organisms but this was also true of other mediums which did not

contain crystal violet. When the contaminating organisms consisted of forms which were not killed or incapacitated by either the 5 per cent oxalic acid solution or the 3 per cent sodium hydroxide solution, they usually grew regardless of the presence of the germicidal dye. The color which the crystal violet imparts to the potato medium provides a contrasting background which enables one to observe minute colonies of bacteria much easier than is true with similar mediums not possessing the dye. This alone justifies the use of the crystal violet in preparing the potato medium.*

<center>COMMENT</center>

Concerning the relative value of the three different culture mediums for the growth of the human and bovine forms of *Mycobacterium tuberculosis* the data obtained from this study seem significant. For the cultivation of the organism when it was of the human type the modified medium of Sweany and Evanoff and the medium of Miraglia gave consistently better results than the medium of Corper and Uyei. Although it is true that growth of comparable luxuriance eventually occurred in most instances on the potato medium of Corper and Uyei, the earlier appearance of the colonies on the medium of Miraglia pointed to the suitability of this medium for the cultivation of the human form of *Mycobacterium tuberculosis.*

These studies confirm the observations of Sweany and Evanoff concerning the advantages of their meat-milk-cream-egg mixture for the isolation of the bovine form of *Mycobacterium tuberculosis* direct from infective material. In addition the value of the modified Sweany and Evanoff medium for the isolation and growth of the human form of the organism of tuberculosis seems

* In my earlier use of the potato medium of Corper and Uyei, some difficulty was experienced in obtaining proper concentration of the crystal violet in the individual portions of potato. Dr. Corper kindly sent me some of the medium prepared in his laboratory and this served as a basis of comparison during subsequent preparation of the medium. Using the medium received from Dr. Corper as a standard of the proper concentration of dye as evidenced by the violet color, further difficulty was not encountered.

established. Apparently the modification used in its preparation did not seriously affect its usefulness.

Almost without exception the bacterial growth which was obtained from the bovine specimens was more abundant on the modified medium of Sweany and Evanoff than that which eventu-
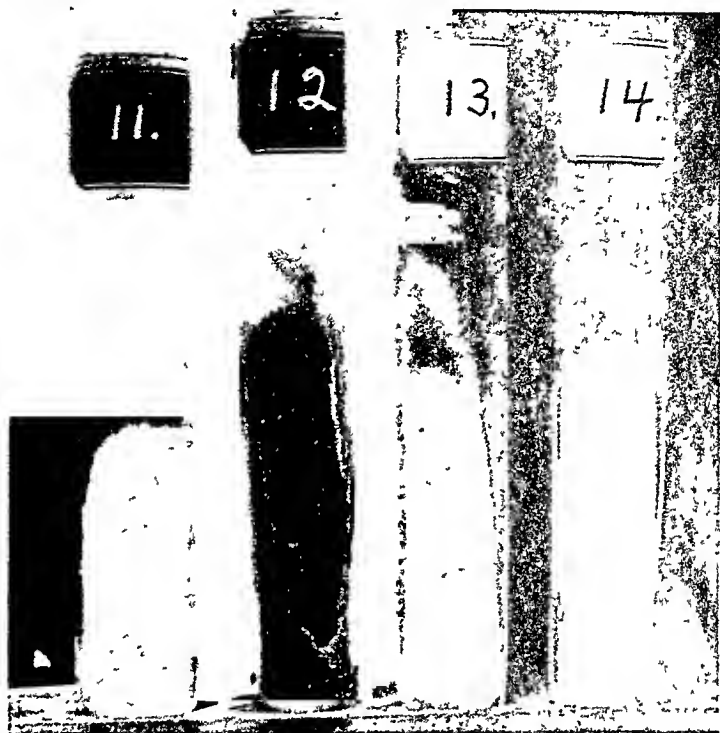

FIG. 1. RESULTS OF ATTEMPTS TO ISOLATE AND GROW THE ORGANISM OF BOVINE TUBERCULOSIS DIRECT FROM AN INFECTIVE LYMPH NODE

Tube number 11 contains the potato medium of Corper and Uyei on which growth did not occur. Tube number 12 and tube number 13 contain the modified medium of Sweany and Evanoff on which growth first appeared forty-eight days after inoculation. Tube number 14 contains Miraglia's medium on which growth did not result. The photograph was taken after one hundred four days of incubation.

ally developed on each of the others. The slowness and infrequency with which colonies of the bovine form of the organism appeared in the medium of Miraglia and of Corper and Uyei would indicate that these mediums do not qualify as satisfactory mediums for this form of *Mycobacterium tuberculosis* (fig. 1).

One can question the desirability of using either of these mediums exclusively for the demonstration of the true character of obscure tuberculous infections in human beings since the work of Aronson and Whitney and of Van Es and Martin, of Griffith and of Mitchell and of the British Royal Commission have definitely shown that the bovine type of *Mycobacterium tuberculosis* not infrequently accompanies human tuberculous infection. In all cultural attempts to demonstrate the presence of the causative organism of tuberculosis in material of human origin the use of the medium of Sweany and Evanoff, besides a medium particularly suitable for the growth of the human form, is desirable.

These data would indicate that the number of *Mycobacterium tuberculosis* present in the respective inoculums probably had a significant bearing on the lapse of time before colonies eventually appeared on the different mediums and on the luxuriance of the resultant growth. Generally speaking, there was a greater number of acid-fast bacilli in the microscopic smears prepared from the sputum and specimens of kidneys than from the specimens of urine, and the inoculums of sputum and of emulsions of kidney yielded acid-fast colonies at an earlier period than the inoculums of urine, in which the number of acid-fast bacilli was much less. This apparent relationship of the number of organisms present in the infective material to the lapse of time which passes before colonies appear on cultural mediums was also demonstrated in another study. Clinical material of human origin was used in which the presence of acid-fast organisms could not be determined by microscopic means. In every instance in which the tuberculous character of the specimen was eventually proved the appearance of the bacterial colonies occurred much later than when inoculums were observed to contain many acid-fast bacilli on examination of an appropriately stained smear.

It is obvious that more luxuriant growth will occur when many bacteria are seeded on a given medium than when only a very few are used. Consequently on suitable mediums one can expect the development of a good growth when many bacteria are present in the material to be cultured and one of less proportions when the inoculum contains a paucity of *Mycobacterium tuberculosis*.

The time which elapsed before colonies of acid-fast organisms developed from the inoculums prepared from the bovine material suggested the great variation in the biologic behavior of the bovine form of *Mycobacterium tuberculosis*. Colonies appeared as early as fourteen days (case 4, table 2) and in several instances their appearance was delayed for as long as eighty-five days. A prolonged period of incubation should be permitted before cultures inoculated with material suspected of possessing the bovine form of *Mycobacterium tuberculosis* are discarded as negative. Even with the modified medium of Sweany and Evanoff, acid-fast colonies were not discernible in one specimen (case 11 table 2) until the seventy-sixth day.

Although it is evident that cultural mediums are available which will promote the growth of most strains of *Mycobacterium tuberculosis* encountered in clinical material, these studies indicate that occasionally a strain may be encountered which stubbornly refuses to produce demonstrable evidence of growth regardless of the kind of medium used. In this connection it may be noted that whereas cultural evidence of *Mycobacterium tuberculosis* was not obtained from certain of the specimens used in this study, definite tuberculous lesions were established in guinea pigs inoculated with portions of the same inoculums from which the cultures were attempted.

Since the true character of the acid-fast organisms which were obtained in these cultural studies was not determined by tests of animal inoculation there is not available absolute proof that each strain secured was *Mycobacterium tuberculosis*. Observations lead me to believe that occasionally cultures of acid-fast organisms morphologically indistinguishable from *Mycobacterium tuberculosis* may be obtained from nontuberculous human material. Acid-fast organisms morphologically like *Mycobacterium tuberculosis* but which are not pathogenic for guinea pigs and rabbits occasionally occur in infective material of bovine origin. Such a culture was recently obtained after fifteen days incubation on an egg medium to which had been added a small amount of an aqueous extract of potato. The appearance of the colonies resembled those of *Mycobacterium tuberculosis* and a stained prep-

aration revealed a pure culture of acid-fast bacillary forms. The injection of rabbits, guinea pigs, and chickens with 1 c.c. portions of a rather heavy suspension of these organisms failed to incite the slightest evidence of tuberculosis. Although it may not be entirely valid to consider infective material of human and of bovine origin as analogous so far as the possession by each of nonpathogenic acid-fast bacilli is concerned, the possibility of bacteria of this character occurring occasionally in human material suspected of being tuberculous seems a reasonable assumption.

CONCLUSIONS

None of the three culture mediums used proved equally efficient for the cultivation of both the human and the bovine form of *Mycobacterium tuberculosis*. For growing the organisms of human origin the modified mediums of Sweany and Evanoff and of Miraglia are considered superior, whereas for the bovine type of the organism the modified medium of Sweany and Evanoff which was slightly modified proved best. This fact would suggest the desirability of using the medium of Sweany and Evanoff in addition to any other which might be used for the isolation of *Mycobacterium tuberculosis* from the infective material of human origin in order to promote the growth of possible strains of the bovine organism which are known to occur.

Definite conclusions cannot be reached as to which of the reagents used is preferable for the elimination of contaminating bacteria. If a sufficient amount of infective material is available it is desirable that inoculums be prepared by both the oxalic acid and the sodium hydroxide method and seeded on duplicate sets of culture mediums.

SUMMARY

A comparative study was made of the efficiency of different cultural procedures for the demonstration of *Mycobacterium tuberculosis* in infective material. A total of twenty-seven specimens of human origin and nineteen specimens of bovine origin was utilized. The culture mediums used were the crystal-violet-

glycerin-water-potato medium of Corper and Uyei; the egg-yolk-glycerin-broth medium of Miraglia and a slight modification of the meat-cream-milk-egg combination of Sweany and Evanoff. Five per cent oxalic acid solution and 3 per cent sodium hydroxide solution were used to control contaminants.

Portions of the material used for the cultural attempts were also used to inoculate guinea pigs and a slightly higher percentage of positive results were obtained from the animal inoculations than from the respective culture mediums. The modified medium of Sweany and Evanoff yielded a greater number of positive results from both the human and the bovine material. In most instances the medium of Miraglia which was inoculated with the human material promoted the growth of acid-fast bacilli earlier than the other mediums used.

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A COMPARISON OF CULTURE AND ANIMAL INOCULA-
TION OF SPUTUM IN THE DIAGNOSIS OF
TUBERCULOSIS

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While working on our concentration method of sputum analy-
sis we[2] encountered a number of patients in whom even by re-
peated concentration examinations, tubercle bacilli were never
found, but roentgenologic and physical findings indicated the
presence of active tuberculosis. These observations prompted
us to undertake a comparative study of microscopic sputum
findings with culture and guinea pig inoculations.

In this series of 200 patients, however, we did not limit our-
selves to those always negative on sputum examinations with
positive clinical and roentgenologic findings. We included in
this series patients with minimal findings; those under observation;
those with glandular tuberculosis; fibroid phthisis, and a few
patients in whose sputums peculiar acid-fast micro-organisms
were found. Also, we have in this series a number of patients
that were positive for some time, but became negative on direct
and concentration examinations, and remained so for at least
nine months.

METHOD

The specimens from these patients were usually so scant that a twenty-four-
hour period was required to obtain a sufficient quantity. This factor enhanced
the finding of bacilli, because we have sufficient data on hand to show that
twenty-four-hour specimens give better results. So, in every instance except
one, work has been done on twenty-four-hour sputum specimens. In this one
exception culture and guinea pig inoculation were made directly from a hilum
lymph node.

Specimens were collected in sterile glass jars, and were incubated for from
sixteen to eighteen hours, in order to obtain a homogeneous specimen for accurate
division. After incubation, if there was enough sputum (7 cc. or more), it was

divided into two equal parts. One was treated with acid, and the other with alkali. But if a specimen was scant, as the majority were, and only one reagent could be used, we invariably used the 3 per cent NaOH, for it gives a higher concentration. To a sputum specimen, therefore, an equal amount of 3 per cent NaOH, (or 3 per cent HCl) was added, the specimen was mixed thoroughly, and incubated for from twenty-five to thirty minutes. After incubation it was neutralized (neutral to pink and blue litmus paper), and the specimen was centrifugated for about twenty minutes. The supernatant liquid was poured off; a smear was made and examined very carefully for the presence of acid-fast bacilli. Only negative sputum specimens were used for the experimental work, with two exceptions, which will be discussed later.

The majority of the specimens came from our Sanitarium, and were, therefore, fresh. Unfortunately, this was not true about the ones that came from Dispensaries, from which deliveries could not be made to the laboratory on the same day they were collected. As a result, cultures of Dispensaries' specimens were contaminated more frequently than the others.

Cultures and guinea pig inoculations were always made on the same specimen, unless specified. The guinea pigs were killed between six weeks and two months after inoculation. The cultures were examined for the growth of tubercle bacilli twice a week for at least two months before they were discarded as negative.

RESULTS OF STUDY

This series of 200 patients may be divided into four groups based on cultural findings and guinea pig inoculations. Out of these 200 patients, forty-nine were children less than fifteen years of age. At present we feel that this group of patients should be studied much more carefully, and guinea pig inoculation should be done not only with the sputum specimens but with feces and throat swabs at the same time.

The first or largest group in this series, consists of 131 patients (65.5 per cent). These patients were found to be negative both on culture and guinea pig inoculations. Of these 131 patients ninety-five were never found positive by any method employed in the laboratory. Thirty-six have been found positive by microscopic examinations, but became negative and remained so even on culture and guinea pig inoculations.

Table 1 gives data on ninety-five patients who were never found positive by any laboratory method.

It is obvious to anyone why most of these patients may become

negative. If there is any question, it may be raised about the negative far advanced patients. This ought to be no mystery, because these patients occasionally become entirely healed as well as those having earlier stages of the disease.

Among these ninety-five patients two deserve special consideration, for in both of these "peculiar acid-fast microorganisms were found."

(1) M. F., Racine Avenue Dispensary No. 26656, was a white male, fifty-two years old, who was first examined in 1928 and was diagnosed as far advanced 'B." Since that time until May 27, 1930, fifteen examinations of sputum were

TABLE 1

CASES NEGATIVE BY EVERY METHOD

DIAGNOSIS	CASES	NEGATIVE EXAMINATIONS
For observation	9	105
Glandular tuberculosis	9	105
Minimum "A"	9	114
Minimum "B"	15	196
Moderately advanced "A"	15	290
Moderately advanced "B"	21	323
Far advanced "A"	5	102
Far advanced "B"	6	110
Abscess	2	104
Bronchiectasis	3	70
Thyrotoxicosis	1	11
Total	95	1,530

made; also a guinea pig inoculation and culture were made on November 17, 1929, all of which were negative. On May 27, 1930 "peculiar clubby acid-fast bodies" were found. Another guinea pig and culture were made on June 17, 1930, but they were also negative.

It is unfortunate that in both these cases we were not able to use the specimens in which the "peculiar forms" were observed for cultures and guinea pig inoculations, and that in the specimens that were used for inoculations no acid-fast forms were found.

(2) R. P., Washington Dispensary No. 26050, was a white female who had been under supervision of the Dispensary since 1908. At that time the case

had been diagnosed as arrested moderately advanced pulmonary tuberculosis. Until 1924 she had been in good condition. In March, 1924 she developed a hemorrhage, but had no record of positive sputum, even at the time of the hemorrhage. On June 14, 1930 "peculiar acid-fast bacilli" were found. (They were very solid and thick, in some places they were finer, but not a single slender typical tubercle bacillus was found.) A twenty-four-hour specimen was obtained, and culture and a guinea pig inoculation were made, with negative results.

Table 1A presents the data on the thirty-six cases in this group. These thirty-six cases, as noted before, were found to be positive but became negative, and all of them save three were negative at least nine months before guinea pig inoculations and cultures were performed on them.

TABLE 1A

Cases Once Positive, Now Negative by Every Test

DIAGNOSIS	CASES	NEGATIVE EXAMINATIONS
For observation...	2	15
Minimum "A"...	9	118
Minimum "B"..	5	62
Moderately advanced "A".............................	4	36
Moderately advanced "B".............................	4	52
Far advanced "A"......................................	7	159
Far advanced "B"......................................	5	74
Total..	36	516

Among these thirty-six patients, three deserve a detailed discussion.

(1) C. R., State Street Dispensary No. 23302, white female fifteen years old. This child was under observation on account of being twenty pounds underweight. Physical findings were practically negative. No roentgenogram was made. On March 24, 1930 two bacilli per slide were found, and on April 11, 1930 another positive twenty per slide was found. (Both positives were found on concentrations.) On May 9, 1930 guinea pig inoculation and culture proved to be negative. Three other microscopic examinations, following guinea pig inoculation, were negative also.

(2) M. N., State Street Dispensary No. 23311, white, male, twenty-two years old, was under observation. On March 18, 1930 the first sputum examination was found positive twenty per slide (concentration). Eight other examinations, all concentrations, were found negative. Guinea pig inoculation and culture made April 9, 1930, with negative result.

(3) R. M., Grand Crossing Dispensary No. 27249, was a white female who had been under supervision since July, 1928. The physical findings at that time revealed a fifteen pound loss of weight, apparent anemia, clubbed fingers, impaired resonance at the right upper lobe, with râles. Twelve sputum examinations were negative. On March 5, 1930 positive sputum, fifteen per slide (concentration) was reported. On June 14, 1930 a twenty-four-hour specimen was obtained for guinea pig inoculation and culture (this specimen was found positive two per slide), but it failed to produce any lesion in the guinea pig, and the culture was negative. Two more animal inoculations and cultures were made, all with the negative results. On September 30, 1930 three specimens were sent in. Two were negative, but the third revealed two acid-fast bacilli per slide (concentration). Two more guinea pigs and a rabbit were inoculated, but results were negative also. The patient is losing weight steadily, and has a moderate cough with expectoration and moist râles in both apices. This

TABLE 2

CASES NEGATIVE MICROSCOPICALLY, BUT POSITIVE ON GUINEA PIG AND CULTURE

DIAGNOSIS	CASES	NEGATIVE EXAMINATIONS
Minimum "A"	1	25
Minimum "B"	1	18
Moderately advanced "A"	3	53
Moderately advanced "B"	4	82
Far advanced "A"	1	18
Far advanced "B"	3	56
Total	13	252

patient apparently has an open case of tuberculosis, but our inability to produce tuberculosis in animals, we fail to explain, unless it is avirulent organism, or an organism that is killed by concentration treatment.

The second group in this series has thirty-three patients (16.5 per cent). Those patients were found positive both on guinea pig inoculation and culture. Of these, thirteen were never found positive before; seventeen were found positive before by the microscopic examinations, but they have been negative at least nine months before guinea pig inoculations and culture were made on their sputum. Table 2 gives information concerning thirteen patients; and table 2a gives data on seventeen patients. Three other patients of this series will be discussed separately.

(1) F. T., Washington Dispensary No. 10094, white male, forty-eight years old, had been under supervision of the Dispensary for ten years. On physical examination he was diagnosed as far advanced. His sputum was never found positive, although thirty-one microscopic sputum examinations were made. On November 23, 1928 his sputum was found positive for the first time, fifteen per slide (concentration). A twenty-four-hour specimen was obtained and was found positive, twelve per slide. From this positive specimen guinea pig inoculation and culture were made. The culture showed food growth of acid-fast bacilli, but the guinea pig showed no evidence of tuberculosis. Another positive specimen was obtained one per field (concentration), from which an animal inoculation and culture were made. The culture produced luxuriant growth; the guinea pig developed an enlargement of spleen and inguinal lymph nodes; smear from which revealed acid-fast bacilli. The Dispensary record shows that

TABLE 2A

CASES ONCE POSITIVE, NOW NEGATIVE MICROSCOPICALLY, BUT GUINEA PIG AND CULTURE POSITIVE

DIAGNOSIS	CASES	NEGATIVE EXAMINATIONS
Minimum "A"	3	52
Minimum "B"	3	43
Moderately advanced "A"	5	70
Moderately advanced "B"	4	78
Far advanced "A"	1	16
Far advanced "B"	1	12
Total	17	271

the patient rapidly grew worse after the first positive sputum was found, and died a month after the second guinea pig was inoculated.

(2) C. B. was a white male, under a private physician's care. He was first seen in August, 1929 when he suffered from massive hemorrhages and acute bilateral pulmonary tuberculosis. His first sputum was submitted on March 13, 1930, and was found positive, four per slide (concentration). The report was held, for two other specimens sent on the same day were negative. Guinea pig inoculation and culture were made on April 9, 1930 from twenty-four-hour specimen. Both proved to be positive.

(3) M. J., M.T.S. No. 28044, a white female, forty-three years old, entered the Sanitarium in March, 1929. Sputum was examined fifteen times, all of them negative. Patient complained of cough with expectoration, streaked sputum, loss of twenty-five pounds in weight, pain in chest, shortness of breath on exertion, and loss of appetite. She had had bronchitis five months prior to this, with a slight amount of yellow sputum, and an "abscess" in the throat a year

before. After admission she gradually became worse, and developed fluid in the chest that was found negative for *mycobacterium tuberculosis* (guinea pig was inoculated). The physical examination revealed impaired resonance at the apices, with no râles. The right base contained fluid. Roentgenogram revealed some dense hilum shadows. All laboratory examinations were negative for tubercle bacilli, or any other definite findings. The patient died on January 17, 1930, and a necropsy revealed a massive involvement of the hilum lymph nodes, with extension along the right main bronchus and up to the trachea. A gross diagnosis of Hodgkin's disease was made, but microscopic section revealed tuberculosis. A guinea pig inoculation and culture that were made from the hilum lymph node revealed virulent bacilli.

The third group in this series consists also of thirty-three patients (16.5 per cent). Those patients were found positive on

TABLE 3

CASES NEGATIVE TO MICROSCOPIC SPUTUM EXAMINATION BUT POSITIVE ONLY BY GUINEA PIG INOCULATION

DIAGNOSIS	CASES	NEGATIVE EXAMINATIONS
For observation..	3	36
Glandular tuberculosis.................................	1	15
Minimum "A"..	1	23
Minimum "B"..	5	81
Moderately advanced "A"...............................	4	79
Far advanced "A"......................................	1	20
Total...	22	341

guinea pig inoculation, only. Out of these thirty-three patients, twenty-two were never found positive before.

Table 3 gives information concerning those twenty-two patients.

Table 3A gives data on eleven patients who were positive by sputum examination once, but have been negative for at least nine months.

In this group, as in the two previous ones, cultures were made parallel with the guinea pig inoculations on all the patients, except one. In that one instance untreated sputum was used for a guinea pig inoculation, and therefore no culture could be made.

This patient was diagnosed as far advanced "B" with tuberculous

laryngitis. He was found positive only twice, and twelve other examinations were negative. We suspected that in his sputum acid-fast bacilli were destroyed by the treatment, so we employed untreated sputum for a guinea pig inoculation. We were successful in producing tuberculosis in a guinea pig, although previous attempt to infect animals with the treated sputum had failed.

In this group, therefore, we have thirty-two patients on whose sputum specimens cultures were made parallel to animal inoculation. Of these thirty-two cultures, ten were contaminated. During the time before cultures could be made different bacteria might multiply in such great numbers that it would be quite often

TABLE 3A

Cases Once Positive, Now Negative Microscopically and Culturally, but Guinea Pig Positive

DIAGNOSIS	CASES	NEGATIVE EXAMINATIONS
Minimum "A"	3	46
Moderately advanced "A"	1	6
Moderately advanced "B"	4	53
Far advanced "A"	1	14
Far advanced "B"	3	57
Total	11	176

impossible to kill them off with our treatment. Elimination of incubation could help in preventing rapid multiplication, and would save time, but we found incubation of a great help in digesting mucoid material in preparing a homogeneous sputum.

The fourth group consists of only three patients (1.5 per cent). These three patients were found positive by the culture method only.

(1) E. M., No. 27334, was a white male, forty-one years of age. Clinically a diagnosis of far advanced "A" was made. Eight sputum examinations were negative. The guinea pig inoculation on December 6, 1928 was negative, but the culture made from the same specimen was positive. The virulence test on the bacilli from the culture was not done.

(2) J. M. M., No. 29310, was a white male forty-nine years old. He entered

the Municipal Tuberculosis Sanitarium in September, 1929. His complaints were loss of weight and productive cough. The diagnosis was moderately advanced "A" with bronchitis. Twenty sputum examinations (10 direct smears and 10 concentrations) were negative. On April 14, 1930 a guinea pig inoculation and culture were made. The guinea pig was negative, but culture revealed growth of acid-fast bacilli, which proved to be virulent.

(3) K. D., No. 29945, white female, nineteen years old. The father and grandfather had died of tuberculosis. Her complaints were loss of weight, slight expectoration, cough, pain in both sides of the chest. There was impaired resonance in the right apex with few râles. Whispered voice was slightly increased. Since January, 1930 twenty sputum examinations were made, all negative. On June 13, 1930 a guinea pig inoculation and culture were made. The culture became contaminated in a few days, so it was repeated on June 22, 1930. This last culture produced a good size colony of acid-fast bacilli, which were found to be virulent for a guinea pig. The guinea pig that was inoculated with sputum on June 13, 1930 showed no evidence of tuberculosis.

The results obtained on this series of 200 selected patients reveals that animal inoculation is still the method of choice in the diagnosis of tuberculosis with certain types of septic material. There are two factors that interfere with accurate culture diagnoses; first, spore forming contaminations, and second, what appears to be weakly protected tubercle bacilli that seem to be killed in the treatment with chemicals. Spore forming bacilli are nearly always present in feces; occasionally in urine, and in most old samples of sputum. In such samples, therefore, it is not safe to risk a diagnosis on cultures alone. Furthermore, we have noticed on numerous occasions some specimens in which treatment has killed the tubercle bacilli, untreated specimens have given growth or infected guinea pigs. Where other bacteria are present, however, cultures are impossible without treatment. Barring these two types, culture work is superior. Its chief advantages are the low cost, and the fact that daily examinations may be made over a long period of time, increasing the chances of finding the bacilli. Furthermore, the value of a positive culture is absolute, while animals are subject to spontaneous infection that occasionally occurs.

We regret that the scant amount of sputum prevented us from using other media, besides cream, for we feel that the comparative study of the different media on this kind of specimens would be

of a real value.   Also, inability to use alkali and acid treatment on all the specimens we feel was a drawback in our cultural work. We have enough evidence from our previous and present work to believe that certain specimens produced growth only when treated by acid, and others by alkali.

From a clinical standpoint, this study reveals that there are a certain percentage of early and moderately advanced cases having clinical disease that apparently never expel tubercle bacilli in the sputum; that there are others which may do so occasionally; and that still others become sputum negative from a few months to years after the beginning of treatment.   The same rule also applies to far advanced patients, except the time limit extends to years and decades.   Many far advanced patients have become negative only after ten or fifteen years of treatment; others become quiescent and become positive from time to time; while others (the greater number) never become negative.

SUMMARY

A comparative study of microscopic sputum examinations, with guinea pig inoculation and culture, were made on selected series of 200 patients, forty-nine of which were children under fifteen years of age.

Twenty-four hour sputum specimens were used for the experimental work, and treated with 3 per cent NaOH.   Half were inoculated into a guinea pig, and half were cultured on the cream medium of Sweany and Evanoff.[1]

This series was divided into four groups, based on cultural and animal findings.

The first, or largest group, consists of one hundred and thirty-one patients, found negative both on guinea pig inoculation and culture.

The second, consisting of thirty-three patients, were found positive both on guinea pig inoculation and culture.

The third group, consisting also of thirty-three patients, were found positive on guinea pig inoculation only.

The last, or fourth group, consisting of three patients, were found positive on culture only.

The experimental work gave us sufficient data to believe that guinea pig inoculation was superior to cultural method for badly contaminated material, but culture study has the advantage of being more absolute, more inexpensive, and permitting of more repetitions.

Atypical acid fast bacilli should be carefully considered but only as suspicious forms until they have produced typical tuberculosis in animals.

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CHOLESTEROL EQUILIBRIUM IN THE LIGHT OF SOME RECENT STUDIES

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I have recently published data on a series of 1,127 valid micropolariscopic examinations of specimens of pathologic urine.[1] These indicated an average incidence of doubly refractive lipoids in pathologic urine of all groups of about 13 per cent. The group of 725 specimens from surgical cases is of special interest. Study of these specimens in relation to involvement of the intestines showed an average positive incidence of urinary lipoids of 21 per cent when the intestines were involved, with a positive incidence of but 8 per cent where there was no involvement of the intestines, as shown in the tabulation.

Through the kindness of Dr. Wm. H. Wilmer, Dr. Warfield T. Longcope, and Dr. Dean Lewis, I have recently been able to check the interpretation of my earlier findings on two patients with gall bladder disease and seven patients with hernia operated on in the surgical clinic of the Johns Hopkins Hospital. Daily specimens of urine, from date of admission to date of discharge, were examined by the micropolariscope in each of the cases. Urinary lipoids were found in every case. The gall bladder cases showed doubly refractive urinary lipoids in 97 per cent of the valid examinations, the finding consisting principally of blastomatous granulo-cellular elements, which I have elsewhere designated lipoid crystals, and of lipoid epithelium.

In six of the seven cases of hernia there was marked displacement of the intestine. In the other case there was severe strangulation and intestinal involvement. This latter case showed presence of lipoid bearing, doubly refractive, round and caudate epithelium as well as the more common squamous lipoid bearing cells. There was a concurrent renal involvement in only one

case, and strangely enough this was the only case examined in which the typical lipoid granule showing characteristic polarizing cross-figure was not found. The specimens from the cases of hernia as a group showed presence of urinary lipoids in 61 per cent of the valid examinations. Fourteen per cent of all the positive findings consisted of the typical granule of characteristic polarizing cross-figure. Thanks are due to Dr. G. G. Finney, Resident Surgeon, for grading the extent of intestinal involvement in the individual cases.

INCIDENCE OF URINARY LIPOIDS FOUND IN 725 SPECIMENS FROM SURGICAL PATIENTS

INTESTINES INVOLVED	per cent	INTESTINES NOT INVOLVED	per cent
Biliary tract surgery	27	Tumors (miscellaneous)	18
Salpingectomies	24	Minor surgery	17
Herniotomies	20	Thyroid surgery	15
Miscellaneous laparotomies	18	Genito-urinary surgery	8
Appendectomies	17	Gynecological surgery	4
		Infections	4
		Eye, ear, nose, throat surgery	4
		Kidney surgery	0
		Traumatic cases	0
Average incidence	21	Average incidence	8

These findings would appear to indicate that in pathologic conditions there may be elimination of lipoids by the kidneys proportional to the extent of the intestinal involvement. They also give added credence to the work of Gardner and Gainsborough[2] who have shown that distinct changes of level of blood cholesterol, as well as changes in its proportional distribution between the free and the combined forms, which may be either up or down, do occur during digestion, and are demonstrable within three or four hours after the ingestion of meals of varying composition.

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GLYCERIN AS AN ADJUVANT TO BACTERIAL DYES

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In every laboratory where bacterial staining is a routine procedure and particularly in those laboratories where such staining is only an occasional necessity, annoyance has been caused by the tendency of certain prepared dye solutions to deteriorate and to precipitate either on the slide or in the bottle. This is especially annoying in the dyes used to perform the Gram stain.

In a search for dye solutions that would not have these characteristics, we hit upon the use of glycerin as an adjuvant. This substance in the proper strength acts, first, as a preservative in helping to keep the dye stuffs in solution, second, apparently as a mordant in increasing staining values, and third, aids by clearing up the background, giving very sharp and clear microscopical pictures.

THE GRAM STAIN

Glycerin crystal violet gram

The solutions are made up as follows:

Mix:
15 parts—3 per cent solution of crystal violet in 95 per cent alcohol.
85 parts—30 per cent solution of glycerin in water.

Counterstains:

A. Red counterstain. Mix 10 cc. of carbolfuchsin with 100 cc. of a 25 per cent solution of glycerin in water.

B. Brown-yellow counterstain. Shake up 2 grams of Bismark brown in 100 cc. of water and filter. To the filtrate add 30–40 cc. of glycerin and mix.

The other solutions employed are the standard Gram's iodine solution and an acetone alcohol decolorizer (75 parts alcohol to 25 parts acetone). Alcohol alone may be employed but decolorization is somewhat slower.

The technique of staining is that of the standard Gram.

1. Cover the film with the glycerin crystal violet solution for one to two minutes. Wash in water.
2. Cover the film with Gram's iodine one minute. Wash in water.
3. Decolorize with acetone alcohol a few seconds until color is practically gone. Wash in water.
4. Counterstain with either the A or B counterstain 30 seconds. Wash in water.
5. Dry and examine.

The above procedure results in microscopical preparations of great beauty, showing a very sharp differentiation against an extremely clear background. The advantages over the usual method are especially apparent in examining smears from exudates where the Bismark brown counterstain is to be preferred.

Variations of the times given are allowable without influencing the results, making it an efficient method even in inexperienced hands.

Using alcohol as a decolorizing agent we have decolorized Gram negative cocci in ten seconds and failed to decolorize *Staph. aureus* in two hours.

TUBERCLE BACILLUS STAINS

The same staining solution (glycerin crystal violet) given above, may be used for the staining of tubercle bacilli in sputum. It is handled in the same manner as carbolfuchsin: steaming one to two minutes or longer; if accidentally boiled, the stain does not precipitate. Wash in water and decolorize by any of the standard methods, wash again, and counterstain with Bismark brown.

The tubercle bacilli appear as slender black rods against a yellow background and are apt to appear thinner and more beaded than with a carbolfuchsin stain.

Although some of those who have seen this stain prefer it in searching sputa for tubercle bacilli, the author prefers the conventional red-blue picture obtained with the carbolfuchsin-methylene blue stains. These preferences seem to be based on a question of eye strain noted where large numbers of slides are examined. One finds as many organisms with one method as the other.

Glycerin carbolfuchsin

We prepared a glycerin carbolfuchsin stain as follows:

Phenol, 5 per cent, 75 cc.
Glycerin, 25 cc.
Saturated solution of basic fuchsin in 95 per cent alcohol, 10 cc.

This combination stains as well as the usual carbolfuchsin, but does not tend to precipitate either in the bottle or on the slide even if boiled. It also gives clearer backgrounds.

GLYCERIN METHYLENE BLUE

A modified Loeffler's methylene blue was made as follows:

Twenty-five per cent solution of glycerin in water, 100 cc.
1 per cent solution of sodium hydrate, 1 cc.
Saturated solution of methylene blue in 95 per cent alcohol, 10 cc.

This mixture stains equally well or better than the usual Loeffler's methylene blue, contains one third the amount of dye stuff and gives sharper pictures.

An ideal blue counterstain for use with the carbolfuchsin tubercle bacillus stain is prepared by adding 2 cc. of saturated alcoholic solution of methylene blue to 100 cc. of a 25 per cent solution of glycerin in water.

The dye solutions given above all keep well, which fact is surprising in the case of the Bismark brown solution but also gratifying since it enables a routine use of this excellent dye.

SUMMARY

We have prepared and used certain modifications of the dye solutions in common employment for bacterial staining in working laboratories and have found that the addition of glycerin not only aids in the keeping properties of these solutions, but that it aids in the staining, greatly increasing the clearness of the microscopical picture.

The Gram method given has been in use in the laboratory of large general hospital for a year with consistent results. This and our own experiences justify us in offering these methods as a help to the laboratory worker.

PATHOGENESIS OF GOITRE*

B. MARKOWITZ

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Consideration of the method whereby pathologic changes occur in the thyroid gland usually results in a goitre classification which is in accordance with the conception of the mode or path the goitre has traveled. The construction of a classification which will be acceptable to both pathologist and surgeon seems rather difficult. This may be explained by the fact that the histologic findings often fail to coincide with the clinical picture. It would be a comparatively simple problem if the different types of goitre were distinct clinical entities and the observations of the pathologist coincided with those of the clinician. The large number of various classifications, however, testify to the lack of agreement as to the interpretation of goitre pathology not only between clinicians and pathologists but also between members of each group. One of the simpler classifications is the one offered by Marine[3] in which he divides the diseases of the thyroid, due to the disturbances of its function, into two groups: (1) thyroid insufficiency and (2) exophthalmic goitre. These two groups are designated hypo- and hyperthyroidism even though they very often overlap.

From the very origin of the embryonal formation of the human thyroid to the time the gland reaches maturity in adult life, we find colloid storage (supply) on the one hand and lack of colloid with hyperplasia (demand) on the other. The maintainence of a balance between these two seems to be the function of this gland. During various periods of life such as pregnancy, puberty and adolescence, the thyroid in man normally changes in texture depending upon the stage of development, and these changes are generally interpreted as due to increased or decreased activity.

With increased activity or greater demand there is, histologically, a marked decrease in colloid containing follicles, a definite increase in the number of cells making up the follicles and general increased vascularity. With decreased activity or supply, the opposite histological picture results: there is a decrease in the number of follicular cells and marked colloid storage in the follicles. The necessity of this simple hyperplasia and its associated increased activity is, most likely, a part of the body mechanism in supplying whatever internal secretion is demanded at that particular period of life and the histological findings of hyperplasia coincide with the clinical symptoms of hypertrophy and increased activity. If left alone this thyroid may revert to normal size and no further clinical symptoms of increased activity may manifest themselves when this particular period of life is passed. The demand which was evidenced by hyperplasia has been supplied and the gland reverts to the normal colloid resting state. There is reason to believe that this normal process is the basis, in its simple form, of disturbed thyroid function resulting in many various stages or so-called goitre types; that goitre production is dependent upon the extreme exaggerations of the normal capacities of the thyroid gland. Chart 1 gives a probable explanation of the route the gland travels in producing various types of goitres.

With the very beginning of thyroid dysfunction it seems probable that hyperplasia fails in its duty of properly supplying the demand and whatever the cause, hyperplasia is increased instead of the gland reverting to the normal resting state. With the ability of adjustment possessed by the animal body it is quite conceivable that the demand may finally be met with a proper supply but only after hyperplasia has progressed to a stage which is above that found in the normally functioning thyroid; and because hyperplasia has already passed the stage of normal, this reversion imitates and simulates, but never reaches the normal. It assumes the resting state as it attempts, but because the hyperplasia has exceeded its normal limits the resting state is now also abnormal in that an over-abundance of colloid has been produced and a simple colloid goitre results.

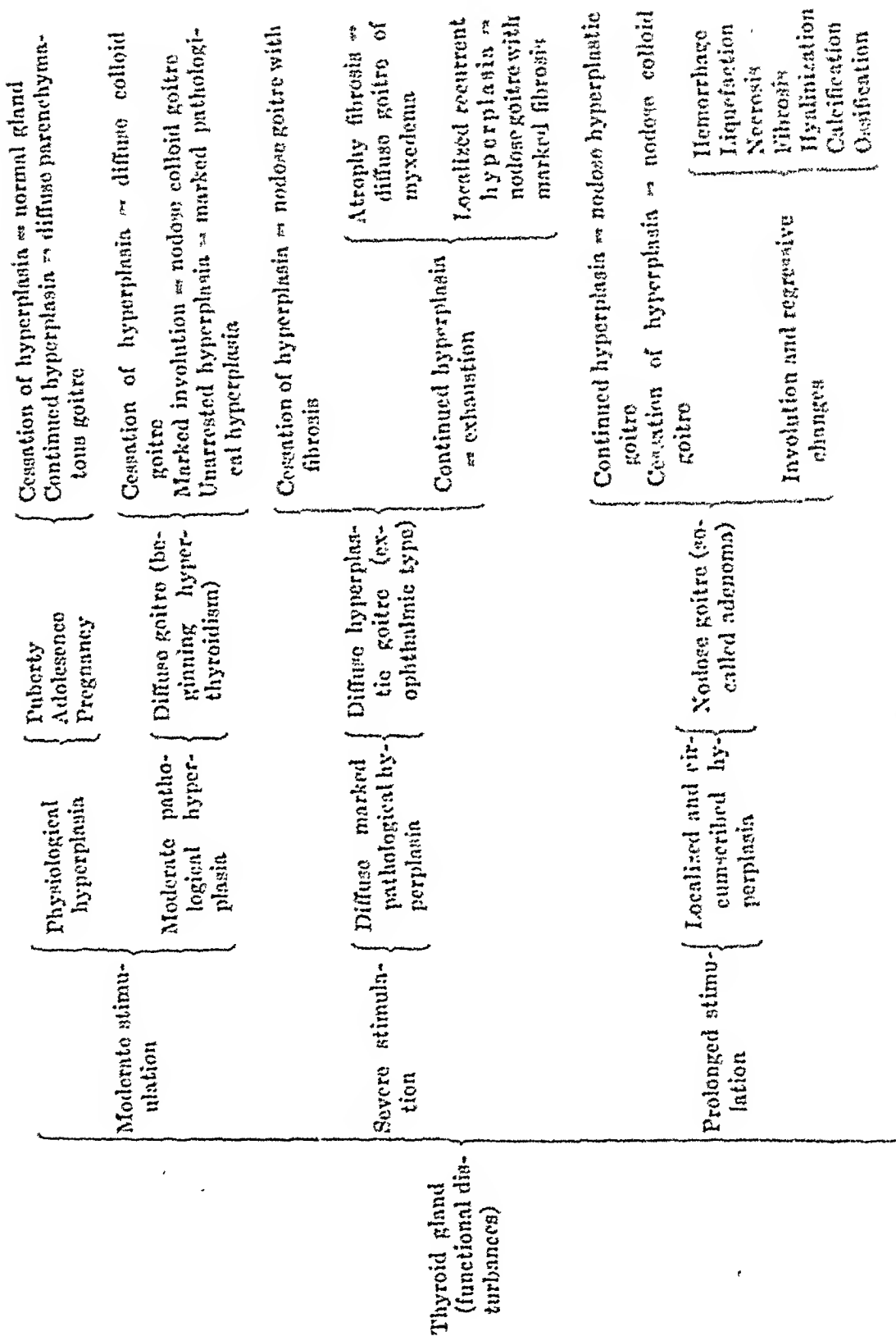Colloid goitre is what the name indicates; an excessive collec-

Thyroid gland (functional disturbances)

- **Moderate stimulation**
  - **Physiological hyperplasia** (Puberty, Adolescence, Pregnancy)
    - Cessation of hyperplasia = normal gland
    - Continued hyperplasia = diffuse parenchymatous goitre
  - **Moderate pathological hyperplasia** (Diffuse goitre (beginning hyperthyroidism))
    - Cessation of hyperplasia = diffuse colloid goitre
    - Marked involution = nodose colloid goitre
    - Unarrested hyperplasia = marked pathological hyperplasia

- **Severe stimulation**
  - **Diffuse marked pathological hyperplasia** (Diffuse hyperplastic goitre (exophthalmic type))
    - Cessation of hyperplasia = nodose goitre with fibrosis
    - Continued hyperplasia = exhaustion
      - Atrophy fibrosis = diffuse goitre of myxedema
      - Localized recurrent hyperplasia = nodose goitre with marked fibrosis

- **Prolonged stimulation**
  - **Localized and circumscribed hyperplasia** (Nodose goitre (so-called adenoma))
    - Continued hyperplasia = nodose hyperplastic goitre
    - Cessation of hyperplasia = nodose colloid goitre
    - Involution and regressive changes
      - Hemorrhage
      - Liquefaction
      - Necrosis
      - Fibrosis
      - Hyalinization
      - Calcification
      - Ossification

CHART I. THE PRODUCTION OF GOITRE

tion of colloid in the follicles with thinning of the interfollicular septa.   The cells lining the follicles are flattened, and the follicles are increased in size.   The picture, in general, is one of rest and decreased activity but yet in certain areas there are small buddings or plications with some hypertrophy of the follicular cells and generalized increased vascularity.   These are most likely remnants of preceeding hyperplasia and testify in favor of increased activity with hyperplasia having been followed by excessive amounts of colloid and storage of this colloid in the dilated and hypertrophied follicles of simple colloid gitre.   Here is seen an overlapping of hypo- and hyperthyroidism; with simple hyperplasia the latter occurs, whereas during the colloid resting state (colloid goitre) the former is found.   Because under certain physiological body changes and at certain periods of life similar hyperplasia is followed by the resting colloid state, it is reasonable to assume that the simple colloid and adolescent goitres are dependent upon the same process which normally operates although in somewhat greater proportions.

In nodular formation it is quite probable that a single localized area of hyperplasia failed to become arrested and to recede to the colloid resting state.   Within this area new follicular structure is formed with increasing lack of orderly arrangement and failure of development of the stroma that characterizes normal thyroid tissue.   Further cellular proliferation within this localized area continues, causing it to increase in size and with increasing growth more and more pressure is exerted upon the surrounding tissue.   In the immediate periphery atrophic changes occur and replacement fibrosis of the portions undergoing atrophy isolates this area of irregular hyperplasia.   As the process continues the the zone of replacement fibrosis becomes compressed and consolidated, completely circumscribing and finally encapsulating a mass of proliferating thyroid tissue.

The development of nodular goitres by this process of increased growth in certain areas, irregular hyperplasia and involution, isolation, complete circumscription and finally encapsulation is borne out by considerable evidence and many investigators support this view (Rienhoff and Lewis,[5] Graham[1] and Hertzler[2]).

That such a process takes place in the thyroid gland is given support by the transition from apparently normal non-nodular thyroid tissue to nodular goitre as seen in the glands of individuals approaching or who have attained adult life. This is particularly seen in females where hyperfunction of the thyroid occurs at certain periods as a physiological phenomenon.

While the colloid resting stage is here an attempt to reversion to normal it has not achieved its object completely, but has reverted to a degree as near normal as possible. Assuming this process to be the one involved, that of simple hyperplasia beginning in the normal thyroid gland which when arrested results in a simple colloid goitre, it must be admitted that there is a possible relationship between hyperplasia, colloid goitre and normal thyroid function.

The ability of bodily adjustment may however fail in some instances and in these cases the intermediate stage of colloid goitre is not seen. The simple hyperplasia of normalcy is not followed by the normal resting stage nor is the hyperplasia slightly in excess of normal, followed by the colloid goitre, but the hyperplasia continues unarrested. The demand apparently cannot be supplied, but nature is not deterred in her efforts to maintain a metabolic equilibrium and the thyroid gland in its attempt to function in its specialized capacity continues its increased activity with resultant diffuse hyperplasia.

One is then dealing with the second type or hyperthyroidism; the goitre with diffuse hyperplasia in which is not found much evidence of the colloid resting stage, but marked activity of the gland. This type is associated with severe constitutional disturbances. If the patient has been known to have had a goitre previous to the onset of symptoms the clinician applies the term "toxic adenoma;" if the patient gives no history of previous goitre the diagnosis "exophthalmic goitre" or "Graves' disease" is applied. Both of these terms serve as an aid and offer an advantage in establishing a clinical entity, but fail in properly classifying the goitre pathologically. The histological picture is essentially one of cellular proliferation and in the advanced case there is actual papillary formation. True one may find many areas in which

the colloid follicles, the contained colloid and the interfollicular stroma are in normal relationhip; a few areas may even show some evidence of the colloid resting state in which the follicles are dilated and filled to their utmost capacity wih colloid. This is merely further evidence that this condition, although it may suggest a separate entity by its clinical manifestations, is only part of some progressive and continuous process.

Should hyperplasia continue unarrested there is a very rapid process with very marked clinical manifestations. The hyperplasia continues diffusely throughout the gland, with absence of involution and resting stage. The onset and continued growth are both very rapid and there seems to be complete absence of thyroid readjustment such as occurs in arrested hyperplasia and involution; a diffuse hyperplastic goitre commonly called Graves' disease by the clinician is the result. In this particular type probably more than in any other, the histological picture is in agreement with the clinical symptoms. The unarrested continued hyperplasia, evidencing marked cellular activity is manifested clinically by the usual symptoms of a very active goitre. The pathologist, even here however, cannot make an interpretation relative to the symptoms which this marked increased activity may produce. For instance, he cannot say whether or not exophthalmus is one of the clinical symptoms, no matter how marked the hyperplasia is on histological examination; nor can he state the degree of toxicity such a histological picture may produce. The terms "exophthalmic," "toxic" and the like are therefore not the best ones to be used in giving a pathological interpretation. Instead I prefer to report the hyperplasia as the essential feature and by way of further explanation state the degree as slight, moderate or very marked and whether or not it is sufficiently marked to produce papillary infolding.

Occasionally the patient may withstand the bodily abuse by the unarrested hyperplastic thyroid and the process continues until there is cellular exhaustion. Without arrest the cells continue in their active hyperplasia until completely exhausted. The high granular active cell becomes flat and inactive and is finally desquamated and absorbed. This is commonly referred

to as the "burned out" gland; while the patient survives, marked pathological changes result with accompanying symptoms of hypothryoidism having been preceded by hyperthyroidism.

At any stage before exhaustion however in this progressive hyperplastic course a readjustment may assert itself and a resting state results. The gland then assumes a resting state, related to, but only as close to the normal as possible, depending upon the degree of the preceding hyperplasia. If the preceding hyperplasia is only of the mild type which produces no clinical symptoms, the arrested state will result in simple colloid goitre which is usually smooth and diffuse. If the preceding hyperplasia has passed this stage and has produced clinical symptoms the arrested state will result in circumscribed areas of colloid with possible nodular formation as compared to the smooth and diffuse simple colloid goitre. The circumscription is due to increased fibrous connective tissue in the interlobular stroma forming connected strands which completely enclose areas of more rapidly growing colloid containing follicles. These nodular colloid goitres differ from the simple colloid goitres only in that the preceding hyperplasia had been farther progressed before it was arrested. When arrest of the hyperplasia does finally occur the process is probably somewhat farther advanced and more intricate than that which results in the colloid goitre. It is highly probable that the hyperplasia continues at least for a longer period, or grows much more rapidly in certain areas with the production of the nodules at these points. Such a picture may be seen in the thyroid of a patient who showed marked symptoms of hyperthyroidism, and who was given iodine for ten days or two weeks prior to operation. If in such a case iodine is continued over a long period of time a diffuse hyperplastic goitre may be changed to a nodular colloid goitre which still shows areas of hyperplasia. Rienhoff[4] demonstrated such involutionary changes from extreme hyperplasia to the colloid resting state. The natural process of readjustment or arrest of the hyperplasia seems to present a similar picture but is apparently much slower in its course.

The cellular proliferation in the gland with or without papillary infolding is usually associated with the symptoms of toxicity

found in the toxic goitres.   In a general way it may be stated that
the severity of the toxic symptoms bears a direct proportional
relationship to the degree of cellular proliferation.   It is a mistake
however to assume that the entire gland of a patient presenting
symptoms of thyrotoxicosis is made up of proliferating follicular
cells.   In many cases the larger part of the cut section is made up
of small colloid follicles and very little active cellular proliferation.
In other cases the larger part of the gland is made up of active
cellular proliferation with marked papillary infolding.   The vary-
ing degree of colloid containing areas in this proliferating type
of goitre may bear some relationship to the other types of goitre
with moderate colloid containing areas.

In studying a large number of goitres one finds that the divid-
ing line between diffuse hyperplastic or exophthalmic goitres and
toxic nodose goitres or the so called toxic adenomata may be only
the route the gland has traveled in this process of hyperplasia-
involution cycles.   Continuous unarrested hyperplasia with little
or no evidence of involution results in diffuse hyperplasia of
the exophthalmic type.   Hyperplasia followed by involution
and constant repetition of this process, of some not definitely
known stimulation, results in the nodular form of goitre, the
toxicity of which may depend upon the degree of suddenly
developed hyperplasia which is not counteracted by involution.
In many of these cases if a careful history is taken, the toxic type
will be found to be an acute exacerbation of an old chronic state
rather than a new or distinct process.   It seems that greater
clarity is achieved by considering this toxic nodular form of
goitre as one of the variations in this continuous hyperplasia-
involution process instead of separating them from pure colloid or
nodular colloid and degenerated goitres.   All goitres may there-
fore be considered as various stages with individual variations of
a single thyroid disease.   In conformity with this conception
the various groups or types of goitre, due to functional disturb-
ances, listed in chart 2 may be looked upon as the various stages
in the development of goitre rather than separate types or kinds
of goitre.

I must add with regard to adenomata that not all thyroid

nodules or nodular goitres found are of the origin here contended. A small percentage, reported by Rienhoff and Lewis series in 109 cases as 8 per cent, are most likely true neoplasms and deserve the term adenomata. Their character however differs from these encapsulated areas here described. The encapsulation does not make them true tumors, but the internal structure does. Micro-

1. Diffuse goitre
    a. Diffuse simple colloid goitre
    b. Diffuse parenchymatous goitre (some forms of adolescence)
    c. Diffuse hyperplastic goitre (exophthalmic)
    d. Diffuse goitre of myxedema (exhaustion fibrosis)
2. Nodose goitre
    a. Nodose colloid goitre
    b. Nodose goitre with fibrosis
    c. Nodular parenchymatous goitre (adenoma)
    d. Nodose hyperplastic goitre (adenoma or exophthalmic or both)

CHART 2. GOITRE CLASSIFICATION

1. True adenoma
2. Carcinoma
3. Langhan's proliferating goitre
4. Benign metastasizing goitre
5. Sarcoma

CHART 3. TUMORS OF THE THYROID GLAND

Primary or secondary inflammation	Thyroiditis (possibility of diffuse hyperplasia-resultant hyperplastic goitre) Strumitis fibrosis Syphilis, tuberculosis, etc. Riedel's strumitis

CHART 4. INFLAMMATORY LESIONS OF THE THYROID GLAND

scopically they show masses of epithelial cells, forming very early follicles which show no lumen; these cells are in strands which anastomose with each other. The blood vessels are quite abundant but no where is there evidence of open hemorrhage. The hyperplasia-involution process is not seen at any stage, nor is there any evidence of lone hyperplasia. These are true ade-

nomata definitely neoplastic in character, and therefore of entirely different origin than the various types of goitre developed through the pathogenesis here described. (See chart 3.)

While infection of the thyroid gland may bear some relationship to the disturbances explained on a basis of dysfunction, we prefer to consider inflammatory disturbances of the gland in a separate class and may classify them as in chart 4.

CONCLUSIONS

In the light of present goitre etiology, I conclude that these colloid nodular goitres or so called colloid adenomata are the result of degeneration dependent upon hyperplasia followed by involution and hyper-involution. That the so called toxic adenomata differ only from the non-toxic colloid nodular goitre in that they contain areas of hypo-involution, in which hyperplasia was not followed by involution. That the so called toxic adenomata are frequently not toxic per se, in which case they are pathologically the same as the non-toxic type, but are associated with hyperplasia in a separate portion of the thyroid not included in the encapsulated nodule.

Further it is highly probable that similar factors are involved in the production of both hyperplastic and colloid types of goitre. That the simple colloid goitre is only an end result of hyperplasia which is mild and not rapid in its course. That the nodular colloid goitre is of similar origin, but differs in that the hyperplasia-involution cycle occurred only in certain areas. That the toxic nodular colloid goitre or toxic adenoma is also of the same origin, but here while the hyperplasia-involution cycle occurs there are a few areas of hypo-involution in which hyperplasia persists. That the true hyperplastic or exophthalmic goitre is one in which there is marked very rapid and unarrested hyperplasia not followed by any form of involution.

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ANTIRABIC VACCINATIONS AT THE CHARITY HOSPITAL OF NEW ORLEANS FOR THE YEARS 1929-1930

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During 1929 and 1930, the Pasteur Institute of the Charity Hospital administered antirabic prophylactic treatment with material prepared as generally indicated by Semple. We desire to present a tabulation of the treated cases, as well as certain statistical considerations thereon and to record briefly methods of vaccine production and to make some observations on post vaccinal results.

PRODUCTION OF VIRUS

All of the vaccine-material used was produced with a strain of fixed rabic virus secured through the kindness of Parke, Davis and Company. Subdural injection of full grown rabbits with emulsions of infected brain stems or cords were accomplished in the usual manner. With the advent of complete paresis, usually on the seventh day after inoculation, the animals were killed by ether narcosis in order to avoid the so common agonal bacterial invasion of the central nervous system noted when such infected animals are allowed to die. The dead animals were immersed in 5 per cent Lysol solution for five minutes, skinned, and again dipped in fresh 5 per cent Lysol solution. After light external flaming, the cord and brain were removed under strict aseptic conditions. Cultures from internal and external portions of the brain and cord were made in 0.05 per cent dextrose broth contained in fermentation tubes. After securing the cultures, an 8 per cent emulsion of brain-cord in normal saline solution was prepared, filtered through three layers of fine linen and 1 per cent carbolic acid added. The carbolized emulsion was incubated at

37.5° for twenty-four hours, after which time it was diluted to 4 per cent by the addition of sterile normal salt solution. Of this emulsion 0.5 mil portions were injected subdurally into each of two full grown rabbits and fifteen glucose broth cultures in five series of five, ten and twenty drops were made. Subsequent similar sub-cultures were made from these original cultures after they had been incubated for five days at 37.5°. If the test animals were living and well three weeks after injection, and if not more than one of each cord-brain culture, and not more than one emulsion culture in any series showed contamination with any type of organism, the material was considered ready for use and a dating of three months from date of carbolisation allowed.

METHOD OF TREATMENT

The following plan of treatment was used, all injections consisted of 2 mil portions of 4 per cent killed virus-emulsion, except for children less than three years of age in whom 1 mil portions of vaccine were injected at a treatment.

Injuries by proven rabid animals

Head injuries. Injections were made twice daily for the first seven days and once daily thereafter for 14 days.

Injuries to trunk and extremities. If multiple and severe, the same treatment was used as for head injuries.

If slight and treatment was begun within six days after injury, treatments were continued for fifteen days, one injection daily.

If slight and treatment was begun more than six days after injury, treatments were continued for eighteen days, one injection daily.

Injuries by unlocated animals

With no suspicious circumstances, fourteen days treatment one injection daily.

With suspicious circumstances, same treatment as for similar type of injury by proven rabid animals.

No actual injury

If rabid or suspicious animals had been handled, eighteen days treatment was given with one injection daily.

TREATED CASES

There were 766 patients treated during 1929, and 467 during 1930. They were classified in the following categories as suggested by the International Rabies Conference of the League of

TABLE 1

CLASSIFICATION BY LOCATION OF INJURY OF 1233 PATIENTS (766 IN 1929, 467 IN 1930) TREATED PROPHYLACTICLY AGAINST RABIES

| LOCATION OF INJURY | 1929 | | | | | 1930 | | | | | 1929–1930 | | | | |
| | Category | | | | | Category | | | | | Category | | | | |
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Head	38*	2	25	7		5		9	7		43*	2	34	14	
Body	23		7	1		2	2	5	2		25	2	12	3	
Superior extremities	161	9	80	17		79	1	40	14		240	10	120	31	
Inferior extremities	133	6	54	12		44		51	11		177	6	105	23	
Multiple sites	11	1	6			19	1	16	11		30	2	22	11	
Totals	366*	18	172	37	172	149	4	121	45	148	515*	22	293	82	320

* One other patient in this category was treated but died.

TABLE 2

AGE CLASSIFICATION OF PATIENTS TREATED

| AGE | 1929 | | | | | 1930 | | | | |
| | White | | Colored | | Total | White | | Colored | | Total |
	Male	Female	Male	Female		Male	Female	Male	Female	
years										
Under 1	0	1	0	0	1	2	2	0	0	4
1–2	21	17	3	1	42	15	5	2	2	24
3–4	54	22	5	4	85	16	15	3	3	37
5–9	108	63	15	11	197	53	49	9	4	115
10–19	86	45	10	4	145	42	40	10	5	97
20–29	42	33	9	12	96	22	26	6	4	58
30–39	34	51	1	4	90	26	29	5	4	64
40–49	26	22	1	5	54	16	12	3	4	35
50–59	16	18	2	1	37	11	9	2	0	22
60–69	6	4	1	1	12	3	4	1	1	9
70–79	2	3	1	1	7	0	1	0	0	1
Over 80	0	0	0	0	0	0	1	0	0	1
	395	279	48	44	766	206	193	41	27	467

Nations in order that the statistics of various institutions may be comparable:

A. Animals proved rabid (microscopic or biologic test).
B. Animals diagnosed as clinically rabid.
C. Animals only suspected (stray, destroyed, or in such a state when received that the brains were unfit for examination).
D. Animals alive and well after an observation period of three weeks, or with negative brains examined after the observation period.
E. Cases undergoing treatment without actually having been bitten (handling positive or suspected rabid animals).

<div align="center">TABLE 3</div>

<div align="center">GEOGRAPHICAL DISTRIBUTION OF PATIENTS TREATED</div>

PARISHES	1929	1930
Allen	2	0
Assumption	2	21
Ascension	15	3
Avoyelles	7	1
Concordia	1	0
Evangeline	2	0
Iberia	1	0
Iberville	1	0
Jefferson	45	50
La Fourche	1	3
Orleans	598	378
Plaquemine	1	0
Pointe Coupee	9	2
St. Bernard.	17	0
St. Charles . .	16	1
St. Helena.	3	0
St. James	9	0
St. John the Baptiste	23	1
St. Tammany	3	6
Terrebonne	4	0 .
Terrebonne .	2	1
Washington .	2	0
W. Baton Rouge	2	0
	766	467

Table 1 records the treated cases in each category together with the types of injury and death. Table 2 indicates the age of treated cases; table 3, their geographical distribution in Louisiana.

TABLE 4

TIME ELAPSING BETWEEN INJURY OR EXPOSURE AND BEGINNING TREATMENT

1929		1930	
Days	Patients	Days	Patients
1	215	Same	8
2	92	1	88
3	79	2	69
4	56	3	54
5	53	4	36
6	58	5	20
7	44	6	20
8	48	7	39
9	23	8	24
10	24	9	24
11	14	10	11
12	7	11	9
13	12	12	18
14	11	13	7
16	2	14	9
18	2	15	5
19	1	16	5
20	1	17	5
21	5	18	1
22	6	22	1
23	4	25	1
24	2	27	10
25	1	28	1
30	3	30	1
31	1	45	1
34	1		
53	1		

TABLE 5

CIRCUMSTANCES OF INJURY AND LOCAL TREATMENT

	1929	1930
Injury inflicted through clothing....................	189	118
Injury inflicted to bare skin..........................	374	201
Tetanus anti-toxin, nitric acid cauterization to site of injury...	386	203
Iodine applied to site of injury......................	43	14
No local treatment....................................	134	102

Table 4 gives the time elapsing before injury or exposure and beginning of treatment. Table 5 indicates the number of injured patients that received local treatment before antirabic vaccination and whether injury was inflicted through the clothing or to the bare skin.

POST VACCINAL RESULTS AND COMPLICATIONS

One patient, seven years old, died of rabies forty-one days after completion of treatment. He had been severely injured about the face by a proven rabid dog and treatment was begun fourteen hours after injury. No post vaccinal paralysis was encountered.

Local reactions at the site of inoculation, usually beginning after the sixth or seventh injection and appearing with from one to ten injections thereafter were noted in 8 per cent of cases. In two cases a disagreeable, generalized, urticarial-like rash, persisting for two or three days and coming on early during treatment, was noted.

DEGENERATIVE LESIONS OF THE APPENDIX (APPENDICOSIS) HITHERTO UNDIFFERENTIATED FROM APPENDICITIS

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During the routine examination of appendices removed at operation certain abnormal changes were observed in some which ordinarily are classified by the pathologist as normal although the clinician observed in the patient, signs and symptoms which were relieved by appendectomy. These observations prompted a systematic study of 1500 surgically removed appendices. The study included the correlation in a selected number of cases of the clinical history, the pathologic findings, the bacteriological examination of the appendicial contents and a follow up of the patients' condition a year or more after the appendectomy. The present report concerns itself with the conclusions derived from the correlations between the clinical manifestations and the pathologic changes.

PATHOLOGY OF RETROGRESSIVE AND DEGENERATIVE LESIONS OF THE APPENDIX

As a result of this study, a group of appendices in which inflammation played no part in the pathological picture was differentiated. The abnormal changes were retrogressive and degenerative in character and terminated in death of tissue. The term appendicosis is proposed to designate the pathologic changes in these appendices. Two types of appendicosis were distinguished. The one essentially retrogressive in nature is characterized by a diminished width of the mucosal layer of the organ, a compression of lymph follicles, a general decrease in the number of mucosal cells and an increase in fibroblasts. Frequently, the usual lining columnar cells are replaced either by cuboidal or flat epithelial

cells. The muscularis may participate in the general diminution of the width of the organ. The lumen of the organ is dilated and almost invariably contains large amounts of fecal material. Occasionally, proximal to the dilated part of the organ there is narrowing or complete stenosis of the lumen. For this type of appendicosis the term pressure atrophy is proposed. It is the belief of the writer that the pressure atrophy type of appendicosis is due to the failure of the appendix to properly expel the fecal
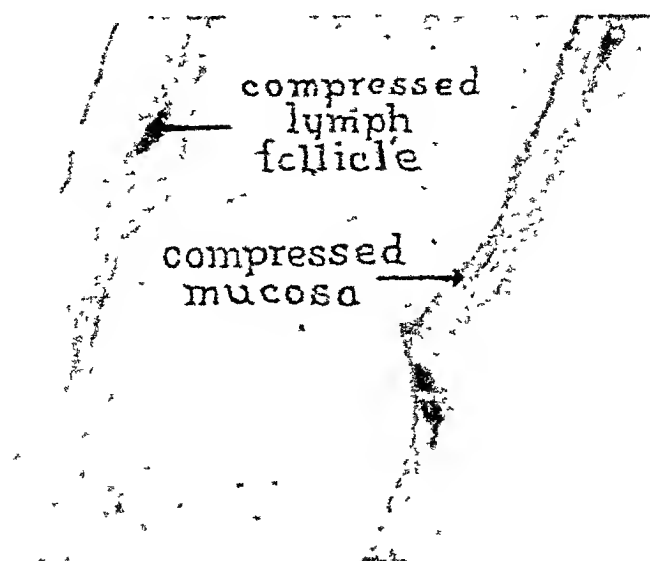
Fig. 1. Appendicosis, Pressure Atrophy Type. (No. 32 Microtessar)

The lumen is dilated. The mucosa is compressed. The lymph follicles are likewise compressed and there is a general diminution of the mucosal cellular content.

contents which continue to accumulate and result eventually in the compression of the wall of the organ. (See fig. 1.)

The second type of appendicosis is degenerative in character and is distinguished by the tendency to formation of erosions. A small area of the lining cells and the mucosa is destroyed and replaced either by blood in lesions of recent origin and by fibres or by connective tissue in lesions of longer duration. These erosions frequently extend through the entire width of the

mucosa to the submucosa.   The erosions may be single or multiple.   In comparatively old lesions the eroded area is surrounded by free and phagocytosed hemoglobin particles.   Not infrequently, the same appendix may show, in other places, evidences of pressure atrophy.   The lymph follicles are usually hyperplastic with large and active germinal centers especially in early lesions.   There is
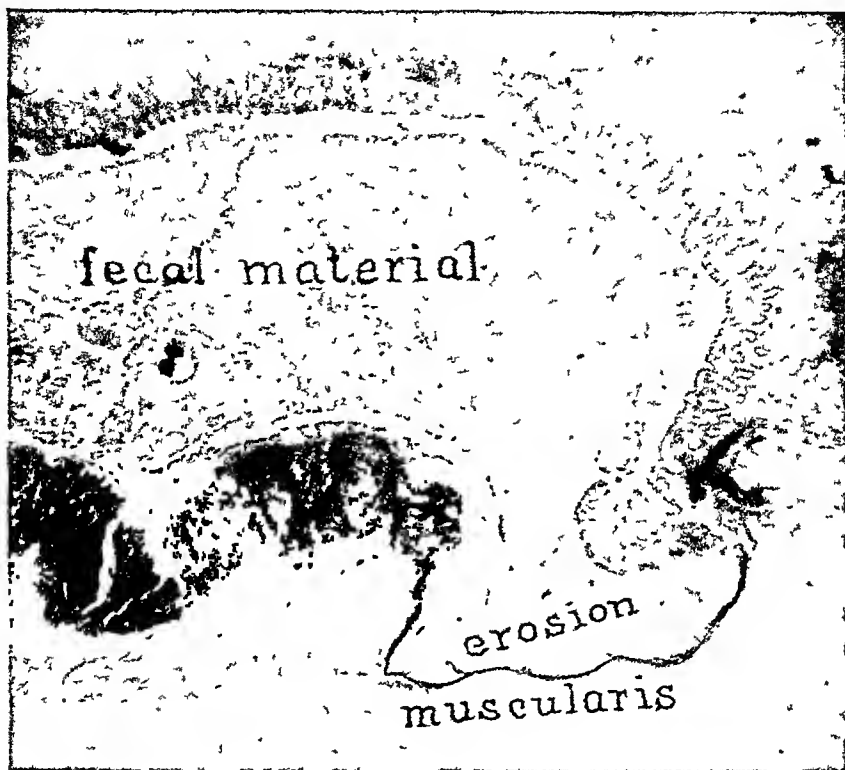
FIG. 2. APPENDICOSIS, MUCOSAL EROSION TYPE (No. 32 MICROTESSAR)

In one place there is destruction of the greater part of mucosa and the defect is filled with material which under higher magnification is demonstrable as blood. The mucosa on either side of the defect contains hyperplastic lymph follicles.

frequently an increase of eosinophiles and large mononuclears in the mucosa surrounding the erosion.   The tubular glands show moderate secretory activity.   The lumen in the location of the eroded area may be very narrow but is occasionally wide. The erosion is most frequently located at the base of a crypt. Erosion type of appendicosis is the term proposed for this form of

appendicial lesion. It is my opinion that the erosion type of appendicosis is produced by sharp particles of feces and the lack of an inflammatory reaction is due either to absence of bacteria or presence of avirulent organisms. (See figs. 2 and 3.)

PATHOLOGY OF INFLAMMATORY LESIONS OF THE APPENDIX

The etiology of appendicitis has been a topic of controversy and speculation since the days of Melier (1827). Fitz (1886) and
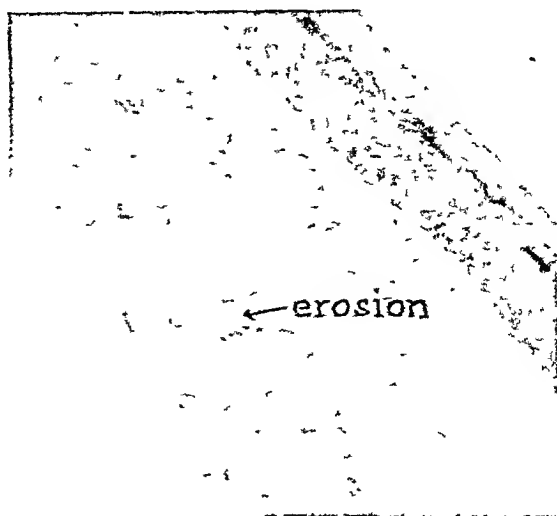
Fig 3. Appendicosis, Mucosal Erosion Type (No. 32 Microtessar)

There is destruction of the entire mucosa and submucosa in one area. The base of the defect consists of heavily stained connective tissue and the defect is filled with fecal material. The mucosa on each side of the erosion shows hyperplasia of the lymph follicles as contrasted with the follicles of the mucosa on the opposite side of the erosion.

Fowler (1894) and remains in the same status today. There are two schools of thought regarding the cause of appendicitis. One is sponsored by Rosenow[2] who is of the belief that bacteria are carried by the blood to appendicial blood vessels where they lodge and set up an inflammation. The cause of the other school is espoused among others by Warren[3] who finds no justification in the belief of the hemotogenous embolic etiology of appendicitis.

It is the general consensus of opinion that the disease is produced ordinarily from an injury to the mucosa and a consequent invasion of virulent bacteria. The hemotogenous embolic route, if a fact, is of rare occurrence. Observations made in this study apparently bear out the conception of mucosal injury and bacterial invasion from the lumen of the organ.

The initial type of lesion noticed in this study was a small ulceration of the mucosa. The lining cells were destroyed and a small area of the mucosa infiltrated by polymorphonuclears. The ulceration may extend through part of the mucosa or to the submucosa. The ulcer may be single without presence of any further evidences of inflammation elsewhere in the organ. This was verified by an examination of longitudinal sections of the entire appendix. Occasionally, the ulcers were multiple. It is assumed by the writer that the mucosal ulcer constitutes the site of the primary injury. The ulcer may heal with consequent fibrosis or the inflammation may extend to the entire mucosa or the whole wall of the appendix. The term appendicitis as introduced by Reginald Fitz admirably fits this type of appendicial disease. For a clearer pathological differentiation I am proposing the following types of appendicitis: (1) mucosal ulcer type in which single or multiple ulcerations of part or the whole width of the mucosa are present, (2) mucosal appendicitis, consisting of a diffuse polymorphonuclear infiltration of the entire mucosa with or without the presence of demonstrable ulcers, (3) diffuse appendicitis in which the entire appendicial wall is infiltrated by polymorphonuclears and other inflammatory cells, (4) gangrenous appendicitis, with or without perforation, where in addition to the diffuse appendicial lesion, there are areas of necrosis which if extending through the entire width of the organ constitute a perforation.

APPENDICIAL CONCRETIONS AND FIBROSIS

Appendicial concretions commonly referred to as fecaliths have been described by many observers. The composition of these concretions has been a subject of much controversy. It is generally accepted at the present that the concretions consist principally of hard fecal material with an occasional central core of some

foreign body. When concretions are found in the lumen of the appendix following an appendicial attack the mucosa usually shows areas of hemorrhage. The lining epithelium in the location of the concretion may be cuboidal or flat in type. There is not infrequently free blood in the lumen. Associated with the concretion may be found any type of appendicosis or appendicitis. Dilatation of the lumen with presence of blood in the mucosa without a mucosal erosion usually indicates that a concretion has been present but was expelled. The term appendolithiasis is proposed for those conditions in which a firm mass of any composition is found in the lumen of the appendix.

There are two views regarding fibrosis of the appendix. One holds that the fibrosis is a senile change, the other that an inflammatory reaction always precedes fibrosis. From this study, it is the view of the author that fibrosis of the appendix follows most frequently the mucosal erosion type of appendicosis and less frequently the mucosal ulcer type of appendicitis. It is not within the scope of this paper to enter into various reasons for this view. The fibrosis observed was either focal or diffuse in type. The focal type is frequently present at the tip of the organ but may involve any part of the appendix with connective tissue replacement of the mucosa and lumen. Occasionally there is stenosis of a part of the lumen with dilatation of the distal or proximal portions. In the diffuse type, the entire appendicial mucosa and lumen are replaced by connective tissue. The terms appendicial fibrosis, focal and diffuse types are proposed for these conditions.

Pathologic classification of degenerative and inflammatory diseases of the appendix

1. Appendicosis
    a. Mucosal erosion type.
    b. Pressure atrophy type.
2. Appendicitis
    a. Mocosal ulcer type.
    b. Mucosal appendicitis.
    c. Diffuse appendicitis.
    d. Gangrenous appendicitis with or without perforation.
3. Appendolithiasis
4. Apendicial fibrosis
    a. Focal type.
    b. Diffuse type.

CLINICAL MANIFESTATIONS OF APPENDICOSIS*

With the pathological classification established, a number of clinical histories were distributed under each of the classifications for correlation. It was found that no clinical differentiation could be made between the mucosal erosion and pressure atrophy types, only the diagnosis of appendicosis could be established. Appendicosis occurred as acute or intermittent attacks extending over weeks, months, or years. One group had continuous symptoms over long periods. The clinician's diagnosis of the cases showing these prolonged clinical manifestations was that of chronic appendicitis. There has been considerable controversy regarding this term. Pathologists are practically in unanimity in denying the presence of such a condition. Aschoff[1] explained the presence of clinical manifestations over long periods by repeated attacks of appendicitis. However, this study reveals that recurrent attacks of an inflammatory disease of the appendix (appendicitis) is not common and that the chronic appendicitis of the clinician is appendicosis of either type or the combined types.

In appendicosis pain was usually dull in character. In patients who had attacks of sharp pain, appendicial concretions were found. There was sometimes slight nausea during the attack. Vomiting occurred very infrequently. There was usually slight to moderate tenderness on deep palpation over the right iliac fossa but rigidity and muscle spasm were absent. There was no fever, the temperature never being found over 99.2°F. The total number of peripheral leucocytes was usually between 8,000 and 9,000 and only occasionally rose to between 10,000 and 11,000. If the appendicosis was intermittent or continuous, patients complained of a more or less continuous abdominal discomfort. There was frequent constipation, lassitude and an indefinite general indisposition.

The clinical picture of a true inflammation of the appendix is so clear cut in the mind of the clinician that it is unnecessary to reiterate the various manifestations. From this study it is the

* I am greatly indebted to Dr. W. M. Stevenson for compiling many of the clinical histories.

belief of the author that one cannot make a distinct clinical differentiation of mucosal ulcer, mucosal or diffuse appendicitis. The clinical manifestations of appendicitis are presented here only as a contrast to those of appendicosis. In appendicitis the pain is usually colicky, or sharp in the presence of a concretion. There is nausea and vomiting. The abdomen over the right iliac fossa is tender to pressure and there is rigidity and spasm. The temperature is slightly elevated and the peripheral total white count is increased, varying between 12,000 and 40,000. In mucosal ulcer types, the symptoms and signs are generally milder in character but mucosal or diffuse appendicitis may simulate the former type. Much has been written in regard to recognition of gangrenous appendicitis with perforation and the author has little to add to this phase of the subject.

Clinical classification of degenerative and inflammatory diseases of appendix

1. Appendicosis
2. Appendicitis
   a. Mucosal ulcer type (questionable of clinical diagnosis).
   b. Diffuse type.
   c. Gangrenous with perforation.
3. Appendolithiasis (clinical diagnosis presumptive only)

SUMMARY

A retrogressive and degenerative lesion of the appendix is differentiated pathologically and clinically. A set of signs and symptoms which had been included under the term appendicitis are differentiated and are ascribed to a pathological entity which is termed appendicosis. A new pathological and clinical classification of the inflammatory and degenerative diseases of the appendix is proposed.

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EDITORIAL

Which Test for Syphilis?

For a quarter of a century the Wassermann reaction has held sway as the most valuable single laboratory procedure for the diagnosis of syphilis in all of its many phases. That it is still of value and the standard by which other tests are judged none will gainsay. However, the many modifications, and the hundreds of "comparative studies" that have been reported have long since shown the nonspecific nature of the reaction, and the fallacy of pinning one's faith to any one technic. In the last few years interesting and favorable reports have been received on various precipitation tests, or preferably, flocculation tests, as the aggregated material that becomes visible in a positive test is not a precipitate from a true solution. From Europe has come the technic of Sachs-Georgi, Vernes, and Meinecke. But investigators in this country are more interested in the Kline, Kahn and Hinton tests. These methods are rapidly gaining in favor also in Europe. The advocates of each test have written voluminously of their comparisons with other methods, all lauding their favorite and basing their conclusions on percentages of agreement, which vary from 70 to 95 per cent, depending on which and how many tests are compared. Let it be admitted that these are all good tests, but that none is infallible. Why not use them all? With 13 cc. of blood, the Kline, Kahn, Hinton and Kolmer tests can all be performed, and the comparison of the results will be of value to the syphilologist, often in those instances in which there is lack of agreement in the results.

The following routine is very useful in effecting a saving both in time and materials. For the preliminary test, before a diagnosis has been made, only 3 cc. of blood are drawn. A Kline test is done at once, using the very sensitive antigen. A negative result with this test, if there is not a history of syphilis, can be taken as

sufficiently final, and further tests need not be done. If the Kline test is positive in any degree, a Kahn test can then be done for confirmation. If this is negative, the clinician must decide whether he wishes further investigation. If the history suggests syphilis, or if both of the rapid flocculation tests are positive, 13 cc. of blood are taken for more complete serologic examination. The very sensitive micromethod of Kline and the Kahn test are again done, and a Hinton test is also set up. The last-named test requires careful technic, and overnight incubation in an accurately controlled water-bath. The reactions with this test are all that are claimed by the originator: they are easily read, are highly accurate, and are persistently positive after treatment and in cases of cerebrospinal syphilis, when other tests on the blood have become negative. Then the old standby, the Kolmer modification, is also set up. The results of all four of these tests are then reported the next day, and the clinician finds his confirmation of his general examination in the agreement, or even in the disagreement, of the results.

A. H. SANFORD.

Clinical pathologists will be interested in an article by W. J. Bell, Deputy Minister of Health of Ontario, on the State's relation to the practice of medicine, which appeared in the Texas State Journal of Medicine, July, 1930. Dr. Bell takes issue with anyone who suggests that preventive medicine is a function of the State and therapeutic medicine is a function of the private physician, claiming that no definite line of demarcation can be properly drawn. State medicine, in his opinion, should be based on whether the whole community is involved in the solution of the medical problem. He admits that for purposes of propaganda the State may well introduce a measure for public health into a community but after that the matter should rest with the private physician.

Two groups of patients, however, must be cared for by the State: (1) Those who are unable to afford medical treatment and (2) those pioneers in a sparsely settled area. However, the medical care of these groups should not slip from the control of the organized medical group and the State should employ the medical profession either individually or collectively to render this service. After setting up these rules, Dr. Bell things that the laboratory branch of medicine should be operated by the State and that for the present the outstanding need is more accurate and modern facilities for diagnosis throughout the medical profession. He states "it is not to be expected that the practicing physician, especially the busy general practitioner, will be conversant with the technique employed in these various laboratory procedures and will be able to perform the tests." He suggests that the State provide at suitable locations a staff for a clinical laboratory center and sufficient number of fully qualified physicians, technically trained and with suitable assistants, and that the initial cost of equipment and housing should be borne by the local community and that the annual maintenance charges

be borne by the central Government and that a minimal charge be made for laboratory services. Service should be rendered only upon written request signed by a physician and reports should be delivered to him, the laboratory in no way assuming direct responsibility for the patient.

The effect of such a scheme is obviously to place the State in competition with private laboratories and even to do away with such laboratories. It would to some extent reduce the need for laboratories in hospitals, although Dr. Bell would carefully guard the interests of the private practicing physician or surgeon. Many clinical pathologists will see in this plan a serious menace to the development of the clinical laboratory, yet the definiteness of the scheme and the fact that to some degree it already exists will warrant its thorough discussion.

Announcement has just been received of the opening, October 1931, of Louisiana State University's Medical Center in New Orleans, to be domiciled in its own quarters. Included in the new Medical Center is Charity Hospital.

Attention of Clinical Pathologists is directed to a letter and blank form, "Survey of Laboratories," which was sent out by the Secretary of the Council on Medical Education and Hospitals of the American Medical Association, under date of May 22.

This communication indicates that the list of pathologists conducting approved clinical laboratory service which was heretofore limited to those who were conducting independent laboratories, is now to be extended to all physicians specializing in clinical pathology and conducting clinical pathology laboratory service whether connected with a hospital or not. Attention is directed to the fact that the American Medical Association has published in a recent issue of the Journal the essentials of an approved clinical laboratory, yet the list of such laboratories as published is a list of pathologists and not of laboratories, the inference being that those pathologists are conducting approved laboratories. One finds, however, that an approved laboratory must be prepared to render service in five different and rather widely separate fields of medicine.

It is quite conceivable that a pathologist might meet with the whole-hearted approval of any examining board, but that he might not be conducting a laboratory which is prepared to give service in more than one or two of these general fields. This usually is true in large institutions where the very nature of the work demands that several men be employed to render adequate service in all of the branches of laboratory work. The laboratories therefore taken as a whole may be quite satisfactory or even superior, but under the manner of listing by the American Medical Association would not properly be listed.

For this reason it is suggested that instead of printing a list of pathologists, a list of approved laboratories be published and the names of those in charge of these laboratories be listed after that of the laboratory. In this way it will be possible to list as a unit the laboratories of a hospital or large institution which may represent the combined group of laboratories being supervised by a number of different pathologists.

It would seem that this would be the only safe way of proceeding, else the American Medical Association will find itself in the position of classifying pathologists as to their relative professional standing.

It is suggested that an expression of opinion on this subject be made directly to the Secretary of the Council on Medical Education and Hospitals.

Clinical Diagnosis by Laboratory Methods. Seventh edition. By J. C. Todd and A. H. Sanford. Pp. 765. 1931. Philadelphia, W. B. Saunders Co. $6.00.

Many new features have been added to the revised seventh edition of this well known book. The greater number of references to the literature will serve as an incentive to the medical student to brouse around and to study more deeply the problems which are of special interest to him. This wider reading often correlates the experimental work with the practical, opening new avenues of interest and giving to the student the fundamental principles of the laboratory test as well as the actual procedure, making the test become something more than routine.

A careful reading of the text indicates that this edition includes many improved methods in all branches of laboratory diagnosis, especially in blood chemistry and bacteriology. The electrolytic Gutzeit test for detecting arsenic and Fairhall's method for lead in the urine are two additions to the subject of urinalysis. Hematology is enriched by including a more elaborate discussion of carbon monoxide hemoglobin giving the technic of Katayama's test, and a brief description of new methods for estimating the hemoglobin content of the blood. A modified method for determining volume index is also added. The discussion of isohemagglutination, including subgroups, diagrams, and the bearing on medical legal cases is more detailed than in earlier editions.

The chapter on chemical examination of the blood contains the greatest number of important changes and additions in the text. A reference to Sander's work on preservation of blood for chemical analysis and the formula for preparing the preservative is an aid to the chemist. The grouping of the tests for non-protein nitrogen, urea, creatinin, and uric acid is more logical and less confusing than that found in earlier editions, and several new methods have been added. There is an interesting reference to capillary microscopy with photographs of capillaries in Raynaud's disease. The Aschheim-Zondek test for pregnancy and the Friedman modification are valuable in view of the importance and reliability of the test.

A. S. Giordano.

Protozoan Parasitism of the Alimentary Tract.   By KENNETH M.
   LYNCH.   Pp. xviii + 258.   1930.   New York, The Macmillan
   Co. $3.75.

The author clearly points out in the introduction that the book
is intended for the medical man who is not too concerned about
the details of protozoology but has to care for sick people who
harbor one or more protozoan parasite, and for the protozoologist
who seeks a connecting link between his science and medicine.
It is not intended that the biologist or the protozoologist will use
it as a reference book.

In the fulfillment of his purpose the author has succeeded, for
the book is a running account of the pathology, diagnosis and
treatment of intestinal protozoa and deals only superficially with
the details of morphology, life history and biology of the various
species.

For the identification of amoebas the author insists on the study
of cysts after pointing out that *E. histolytica* has four nuclei and
E. coli eight.   He states, "this simple mathematical proposition,
so difficult to get across to medical laboratorians, is the most impor-
tant thing in the diagnosis of amoebiasis, it is the sole conclusive
point in the chronic case, practically considered."

This is a statement with which many will disagree and on
perfectly sound ground.   The *structure* of the nucleus is far more
important than the number of daughter nuclei and therefore if
cysts are to be used for identification, nothing short of iron-hema-
toxylin staining is final.

On the whole the author's views on the rôle of protozoa in the
cause of disease are conservative and he does not agree with cer-
tain enthusiasts who are all too eager to place upon the delicate
shoulders of any protozoan the cause of any symptoms or illness
of a given patient.   His discussions on treatment are also conser-
vative but summarize the best knowledge on the subject.

The book shows careful topography, the illustrations are cer-
tainly excellent and it is recommended to all interested or con-
cerned with these protozoan parasites, for the frank discussion of
the subject by Dr. Lynch will do everybody good even if the
reader does not agree with all the author's conclusions.

THE PRINCIPLES OF VACCINE THERAPY*

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There is no subject in biologic therapy quite so difficult of evaluation as vaccine therapy and especially in relation to the treatment of disease. A review of the very extensive literature accumulated during the past twenty-five years leaves one greatly confused and with the impression that the attitude of the physician toward vaccine therapy has frequently influenced the results reported. In other words some physicians appear to have secured uniformly good therapeutic effects while others have observed poor and indifferent results with approximately similar vaccines and technic. This psychological factor apparently exerts an important influence among the laity in that those who have great faith in vaccine therapy almost invariably secure better real or imaginary therapeutic results than those who have little or no faith in this form of treatment. This trust is not infrequently justified by the confidence instilled by those physicians whose results are much superior to the average because they have superior knowledge of methods for preparing and administering vaccines.

FIRST PRINCIPLE

This difference in results and opinions refers almost entirely to the use of vaccines in the treatment of disease. In the prevention of certain diseases, notably of smallpox, rabies, typhoid fever, anthrax and diphtheria, methods of active immunization have yielded such uniformly excellent results that there is a remarkable unanimity of opinion on the value of vaccine therapy constituting

* Read at a Symposium on Vaccine Therapy before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

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the first principle namely, *that vaccines have proven of more value in the prevention than in the treatment of disease.*

SECOND PRINCIPLE

That vaccines continue to be used in the treatment of disease despite the poor technic and abuses committed in their use during the past twenty-five years indicates that there must be something of real merit in this form of therapy. Within recent years certain distinct improvements in the methods of preparing stock and autogenous vaccines, with special reference to the utilization of the soluble or exogenous products and toxins of micro-organisms and the abandonment of heat for purposes of sterilization, have apparently improved the results of the vaccine treatment of some diseases as compared with those employing the orthodox heat killed suspensions so that the second principle is *that the method of preparing stock and autogenous vaccines appears to have an important bearing upon their therapeutic value.*

THIRD PRINCIPLE

The maximum of specific effects in active immunization depends to a large extent upon incorporating in the vaccine the important organism or organisms of primary infection rather than those of secondary infection and importance. In other words it may make little or no difference how much skill and care are exercised in the preparation of a vaccine, if the correct and important organism or organisms are absent the maximum of specific effects can not be obtained. For this reason the first essential for success consists in accurate bacteriological diagnosis, not only for the proper selection of a stock vaccine but more particularly for the proper preparation of an autogenous vaccine. Therefore the matter of success or failure in the vaccine treatment of disease rests primarily in the hands of the one making the culture with special reference to the choice of medium and the manner or method of securing and culturing the material. For example, cultures from a discharging ear on plain agar may cultivate only staphylococci or diphtheroid bacilli of secondary infection and miss entirely a primary streptococcus or pneumococcus. Under these conditions a vaccine pre-

pared with the greatest skill may be expected to yield at best only some degree of non-specific effect. Ignorance or carelessness in these particulars may defeat the purpose of vaccine therapy at the very outset and for this reason I believe it may be stated as an important third principle *that successful vaccine therapy demands accurate bacteriological diagnosis and especially the employment of proper and acceptable methods for securing the important organism of infection for the preparation of autogenous vaccines.*

FOURTH PRINCIPLE

Owing to the fact that some species of bacteria exist in more or less well defined, immunologically specific strains and especially in pneumococci, streptococci, meningococci, staphylococci, gonococci, typhoid bacilli and possibly others, it would appear as a fourth principle *that well prepared autogenous vaccines are to be preferred to stock vaccines in the treatment of disease.* It would also appear that stock vaccines for prophylactic immunization should be polyvalent whenever evidence indicates the existence of immunologically specific strains. It is true that polyvalent stock vaccines may meet this principle for the treatment of disease but the fact remains that autogenous vaccines are to be preferred from this standpoint and offer the added advantage of freshness of preparation with possible greater vaccinogenic activity.

FIFTH PRINCIPLE

Since available evidence indicates that living bacteria possess the greatest degree of antigenic activity, it would appear as a fifth principle *that, whenever possible, vaccines should be composed of living organisms of reduced or modified virulence or so prepared as to approach this state as nearly as possible.* For example cowpox vaccination against smallpox still stands as the most efficient example of active immunization because a modified, but living, virus is employed. The same is true in Pasteur's original methods for vaccination against rabies and anthrax. Indeed, the only hope for developing a method for immunization against tuberculosis would appear to reside in the use of living vaccines of tubercle bacilli of reduced virulence as employed in recent years by Cal-

mette and Guérin. For obvious reasons living vaccines are of very limited application but I believe that the principle involved is well defined. For this reason vaccines should approach this state as nearly as possible with the minimum of heat and other technical procedures in their preparation.

SIXTH PRINCIPLE

Since it would appear that the soluble exogenous products and toxins of bacteria rank very high in vaccinogenic activity, I believe that it may be stated as a sixth principle *that these toxins with or without modification should be always incorporated or used whenever possible in the preparation of vaccines.* As examples may be mentioned the notable success of vaccination against diphtheria with toxin-antitoxin and toxoid as well as the measure of success attending vaccination against scarlet fever with the toxin of *Streptococcus scarlatinae* and the greatly improved results in the treatment of staphylococcus infections with the toxins of *Staphylococcus aureus* commonly designated as "filtrates." Furthermore it is well known that the soluble toxins act as the most antigenic agents in the immunization of horses as shown by the success attending the preparation of antitoxins for diphtheria, tetanus, gangrene, scarlet fever, botulinus, and so forth. Since it would appear that the majority of pathogenic organisms are apparently capable of producing some amounts of soluble toxins in fluid culture media of proper hydrogen ion concentration and preparation, it would appear that methods of preparing vaccines should be changed to permit the inclusion of as much toxin as possible.

SEVENTH PRINCIPLE

From this standpoint heat should be used less frequently or abandoned in the sterilization of vaccines and especially in the preparation of staphylococcal vaccines and others in which the toxins are thermolabile. Sterilization with chemical agents like tricresol or phenol appears to produce less destruction of these very desirable vaccinogenic agents so that it may be stated as a seventh principle *that in general terms the sterilization of vaccines with chemical agents or sterilization by filtration are*

to be preferred to sterilization by heat. The method to be employed depends upon the source and nature of the toxin; for example, both heat and chemical agents destroy staphylococcal toxins so that these vaccines are best prepared by filtration alone whereas the vaccinogenic activity of other toxins converted into toxoids by chemical agents appears to be maintained in some degree as in the case of the toxoid of diphtheria toxin. Possibly the best type of vaccine to be employed in the prophylaxis and treatment of disease is that incorporating the soluble toxins along with chemically killed organisms although in the case of diphtheria the toxin alone may be employed because it so nearly constitutes the sole pathogenic agent in the production of this disease.

For several years I have employed vaccines of this kind which may be prepared by cultivating the organism in a suitable broth medium for five days followed by sterilization with tricresol and dilution to the desired numerical strength. In the preparation of staphylococcal vaccines however, in which the action of chemical agents upon toxins is to be avoided, the culture is filtered through a sterile Berkefeld filter and the desired number of separately prepared, chemically killed cocci are added to the filtrate.

Since toxins tend to deteriorate, freshly prepared autogenous vaccines are likely to be more vaccinogenic than stock vaccines of varying age kept at varying temperatures. Furthermore since inflammatory exudates like bronchial secretions are likely to contain soluble toxins, it is to be expected that vaccines composed of the sterile filtrates of such exudates may possess definite vaccinogenic and therapeutic value as indicated by the reports of Eiman and others.

EIGHTH PRINCIPLE

From the standpoint of prophylactic immunization it would appear that results are very largely dependent upon the production of specific antibodies whereas in the treatment of disease certain non-specific effects of therapeutic value may develop so that it may be stated as an eighth principle *that prophylactic immunization is largely dependent upon specific antibody production requiring varying periods of time for the production of adequate*

amounts of antibodies whereas in therapeutic immunization a part of the effect may be more immediate and due in part to non-specific agencies produced by the proteins of the bacteria and culture medium.

NINTH PRINCIPLE

But beyond reasonable doubt the maximal beneficial results possible in prophylactic and therapeutic immunization would appear to be due to antibody production and in this connection the route of administration, dosage and intervals between injections are of importance, especially in the vaccine treatment of disease, and possibly account in part at least for the widely varying results attending vaccine therapy.

From this standpoint the value of the skin as an immunological organ of importance in relation to antibody production commands more attention at present than hitherto and I am disposed to state as a ninth principle *that the intracutaneous injection of vaccines is of more prophylactic and therapeutic value than subcutaneous injections.* At least certain studies by Tuft and others have indicated that more antibody is produced by the former route of administration although the doses must be smaller, but the larger local reactions may constitute an objection to this route of administration. I believe however, that we do well to use intracutaneous injections more frequently than is customary, especially in the treatment of disease. Indeed present and future investigations may show that a particular route of administration may be required for securing the maximal vaccinogenic effects of certain bacteria, the immunizing capacity of pneumococci by oral administration being an example as shown by the recent studies of Ross.

TENTH PRINCIPLE

In this connection Besredka's method of immunization by the local or topical application of vaccines commands a good deal of attention from the standpoint of utilizing the possible antibody producing capacity of the tissues, skin and mucous membranes to a greater extent than is the case when vaccines are given subcutaneously or intramuscularly at a distant focus and on the assumption that antibodies will be produced and conveyed to the infected

tissues by way of the blood or lymph. It is true, however, that the results reported from the use of Besredka's "antivirus" or vaccine by local application may be due to the presence of bacteriophage in the filtrates although I believe that the possibility of therapeutic effects from the local production of antibody is possibly more important than the possible destruction of microorganisms by bacteriophage, especially if the matter of concentration of the latter is of prime importance as indicated by the recent investigations of Krueger and Northrup.

On the other hand, it would appear that the maximal degree of non-specific effects from vaccines is elicited by intravenous injection in small doses and that second in efficiency is the intramuscular or subcutaneous injection of relatively large doses. For this reason the non-specific protein treatment of disease with stock or autogenous vaccines is best carried out by the intravenous route of administration so that it would appear as a fairly well defined tenth principle *that the route of administration and dosage of vaccines have some bearing upon the results and these should be selected according to the indications and requirements of individual cases.*

It is difficult to furnish definite directions for the administration of vaccines by inexperienced physicians, which constitutes a reason for no small part of the widely varying results observed by physicians in the practice of vaccine therapy and properly accounts for the growing custom of entrusting vaccine therapy to those specializing in this field of therapeutic science.

ELEVENTH PRINCIPLE

There is still such a wide diversity of opinion on the value of vaccines in the treatment of acute bacterial infections that it appears impossible to evolve at present a principle on this phase of the subject. That the administration of a small dose of vaccine materially adds to the burden of toxemia in acute infections can be excluded as a possibility of importance and constitutes an objection of little or no weight. But that the administration of a vaccine in acute infections may result in an over-stimulation of immunological defense is a matter of more importance. Whether the usual assumption that an individual with an acute infection is

undergoing as much immunological stimulation as can be borne
or is safe is, however, open to question because of the possibility
and probability that antibodies are produced by the tissues
directly involved in infection and that the administration of a vac-
cine may bring into play the immunizing capacities of tissues not
otherwise materially involved in antibody production.

My clinical experience has taught me that the administration of
small doses of vaccine is frequently of value in the treatment of
some acute infections even including the surgical septicemias, so
that I believe it may be stated as an eleventh principle *that vaccine
therapy is sometimes of value as part of the treatment of acute infec-
tions.*

TWELFTH PRINCIPLE

Vaccines probably have their widest field of application in the
treatment of chronic infections where the stimulation of antibodies
or the production of fever, leukocytosis and other non-specific
effects are likely to be of particular value.   However, opinion of
their value in such cases is subject to great variation and a wide
impression prevails that vaccine therapy has not by any means
fulfilled original expectations and that the results reported by
enthusiasts can not be generally verified or duplicated.   So
many factors of importance are involved from the method of mak-
ing the culture for bacteriological diagnosis and securing the right
organism or organisms for the preparation of vaccines to the
method of preparing and administering them, that it is well to
keep an open mind on the subject and pay more attention to ways
and means for detecting the reasons and sources of success and fail-
ure with proper measures for correcting the latter.   Therefore I
believe it may be stated as a twelfth and final principle *that
vaccines in the treatment of some chronic diseases have met with a
measure of success and are worthy of further use especially by those
possessing special skill and experience and with due regard for im-
portant technical details involving their preparation and administra-
tion.*

SUMMARY

In conclusion, these twelve principles may be summarized as fol-
lows with the hope that they may arouse renewed interest in vac-

cine therapy and on the assumption that they reflect in a general manner the present status of this important subject:

1. Vaccines have proven of more value in the prevention than in the treatment of disease.

2. Methods of preparation of stock and autogenous vaccines appear to have an important bearing upon their therapeutic value.

3. Successful vaccine therapy demands accurate bacteriological diagnosis, especially the employment of proper and acceptable methods for securing the important organisms of infection for the preparation of autogenous vaccines.

4. Well prepared autogenous vaccines are to be preferred to stock vaccines in the treatment of disease.

5. Whenever possible vaccines should be composed of living organisms of reduced or modified virulence or so prepared as to approach this state as nearly as possible.

6. Bacteriological toxins with or without modification should always be incorporated or used whenever possible in the preparation of vaccines.

7. The sterilization of vaccines with chemical agents or by filtration is to be preferred to sterilization by heat.

8. Prophylactic immunization is largely dependent upon specific antibody production whereas therapeutic immunization is probably due in part to both specific and non-specific effects.

9. The intracutaneous injection of vaccines may be of more prophylactic and therapeutic value than subcutaneous injection.

10. The route of administration and dosage of vaccines have some bearing upon the results and should be selected according to the indications and requirements of individual cases.

11. Vaccine therapy is sometimes of value in the treatment of acute infections.

12. In the treatment of some chronic diseases vaccines have met with a measure of success and are worthy of further use especially by those possessing special skill and experience and with due regard for important technical details involving their preparation and administration.

A CONSIDERATION OF THE THEORETICAL BASES FOR VACCINE THERAPY*

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Any discussion of the subject of vaccines is in danger of becoming prolix by virtue of the diversity of ideas bound up in the word itself. It is in fact noteworthy that whereas in immunology we are plagued and at times confused by the very multiplicity of terms; we have failed to adopt a useful word to substitute for the improperly used vaccine. While at first it might appear that we could do very well with a general understanding that the term vaccine is incorrectly used to cover any antigen used specifically and parenterally for the purpose of conferring immunity, it is consistent with such a procedure to include toxins in such a category and to exclude such agents as Besredka's antivirus. It would appear then that one of the contributions of this symposium might well be an attempt to provide a satisfactory substitute for the word vaccine.

In view of the laxity of the terminology, it is necessary to introduce this paper with the statement that it is concerned only with the prophylactic and therapeutic use of killed bacterial antigens when administered parenterally.

In looking over the literature on this subject, one is struck by the observation that the theory of vaccination practically ceased to develop after the work of Wright and Douglas on opsonins. Since the early days of the century, practically the only justification for the practice of vaccination has been clinical or statistical in nature. Had clinical experience been uniformly favorable, had statistics been unchallenged, and had immunology as a science

ceased to develop there would be no occasion to do more than to
set aside this time in which to eulogize vaccines.   If on the other
hand theory has lagged, clinical experience failed or statistical
evidence faltered, there would be occasion to take account of
stock.   The fact that we are met is indication that one or all of
these situations exists.

The theory of vaccination is essentially this: that parenteral
introduction of killed bacterial antigens induces the formation
of specific antibodies which in turn neutralize or combat present
or succeeding infections with bacteria of the type used in the vac-
cines.   The antibodies involved are agglutinins, precipitins,
opsonins, complement fixing and bactericidal antibodies, and to a
limited extent antitoxins.   If there are others, they are more or
less obscure and limited in application.   It is essential in evaluat-
ing vaccines that we accept this theory, advance another or fall
back upon clinical and statistical observations as the sole support
for our practices.   It is my purpose to indicate the extent to
which theory has suffered as a result of modern research in both
bacteriology and immunology.   This can be accomplished in two
ways; first, by considering the ability of specified antigens to
produce suitable antibodies in vivo, and second, by appreciating
the probable rôle of these antibodies in preventing and remov-
ing infection.

That a given killed organism can produce all of the antibodies
mentioned above is conceded, and so long as only quantitative
differences in separate antibodies could be observed, it was logical
to conclude that the antibody thus produced could react with the
antigen in the form of the living bacillus.   If, however, we permit
ourselves any doubt as to the essential identity of the two antigens
(living or killed) or if the possibility arises that the agent of infec-
tion is different from the organism used as a vaccine, a marked
change in procedure is indicated.

While there is and will be for a long·time much controversy
concerning the extent and significance of so-called bacterial varia-
tions or dissociations, there is a general acceptance of some of the
more obvious features.   For example, it is now recognized that
many if not all organisms contain at least a dual antigen.   Weil

and Felix[13] were the first to observe the duality of antigen in a single organism. Working with Proteus X 19 and blood from typhus patients they observed qualitative differences in agglutination reactions. They observed that the proteus strain was made up of two antigens, one heat stable, the other heat labile; one produced agglutinins giving a heavy flocculent precipitate, the other agglutinated in fine, slowly settling particles. Felix[2] extended this work by showing that other organisms possessed these two antigens. He went further and claimed that the natural disease (typhoid fever) resulted in the formation of fine agglutinins, whereas inoculation with heat killed antigens produced only the large flaking agglutinins. From this Felix argued that immunization as now practiced has no preventive power since it does not produce reactions equivalent to those resulting from the natural infection.

The fundamental observations of Felix have been amply confirmed although his claims concerning the futility of vaccination have certainly not affected vaccination procedures.

Investigations in the field of microbic dissociation are no less interesting and suggestive. In spite of the extreme claims of certain bacteriologists, there has emerged from studies of the variability of microörganism certain well defined and largely accepted concepts. Among these are the following:

Bacteria are capable of variation within limits characterized by two extreme forms, the so-called S and R types. The S or smooth form is associated with infection, is characterized by maximum virulence and is identified by its smooth, regular colony (with certain exceptions). The R or rough form is obtained by prolonged culture on artificial media or under unfavorable conditions. It is avirulent or has a reduced virulence and grows in a peculiar irregular granular colony. Of greatest interest from the standpoint of vaccination is the behavior of these types when grown in serum produced by the opposite type. Soule[12], Hadley[7] and others have shown that the rough avirulent organism when grown in an antiserum produced by the smooth virulent form becomes itself smooth.

Having confirmed these observations, I[9] investigated the cul-

tures used in the preparation of typhoid vaccine in several leading laboratories and found that in general an R type predominated. The danger of producing sera in inoculated individuals capable of changing avirulent into virulent organisms is obvious. Some time later Grinnell[6] studied the bactericidal power of blood obtained after immunization with smooth and rough strains. He concluded that if bactericidal power of the blood had any value as a measure of immunity, it was decidedly necessary to use a smooth type organism in preparing vaccines. These observations have already had their effect upon routine procedure in the production of vaccines.

The effect on the theory of vaccination of these developments in bacteriology, although seemingly slight, is in fact considerable. So long as there existed only a vague idea that all members of a given bacterial species might not be alike, there was little reason for altering either ideas or practices. As soon, however, as it was definitely shown that bacteria were not only different at varying periods of growth, but that bacterial antigens were materially altered in the process of vitiation, the idea that antibodies resulting from inoculation of killed bacteria were identical with those resulting from infection could not be sustained.

The case for autogenous vaccines is materially strengthened by these observations for here at least antigens are most likely to be of the S type. But this advantage is reduced by using heat to kill the organisms. In fact any process that results in a denaturization of the bacterial proteins must now be regarded with suspicion. Realization of this fact has helped in the development of such substances as antivirus, bacteriophage, and immunogens, vaccines supposedly containing the bacterial proteins in their native state.

The effect of these advances in knowledge of the antigenic structure of bacteria on the theory of vaccination is somewhat devastating. If it can no longer be assumed that the antibodies produced by heat killed vaccines are identical with those produced by living organisms, we are tempted to discount the rôle of antibodies in immunity. This, as a matter of fact, we have been doing for several years.

There are few if any modern immunologists who believe that agglutinins and precipitins play any significant rôle in immunity. The same cannot be said for the opsonins and bactericidal antibodies. It is well known that blood has bactericidal properties and that the leukocytes have power to ingest bacteria. It is not at all difficult to believe that both of these abilities are factors in immunity. So far, however, I have not succeeded in finding studies showing an increase in these so-called antibodies during recovery from disease. It has been demonstrated that bacteriolysins and opsonins are decreased during an acute attack and it appears that following recovery they return to the level generally considered normal. So far as bacteriolysins are concerned, experience has shown that vaccines do not cause them to increase. On the other hand, Grinnell's[6] work previously cited would indicate that the difficulty was with the vaccines.

But the opsonins are our chief comfort and they remain today almost the sole theoretical basis for the use of vaccines in spite of the fact that the testing of sera for opsonic power has become almost a lost art. If we are content to rest our case for vaccines, at least so far as theory goes, on the record of opsonic indices, it is quite in keeping with a sceptical mood to examine the records obtained by various means and especially to compare results obtained by the use of the traditional vaccine and by some of the newer substitutes. This field has scarcely been scratched experimentally and the only facts available concern the enormous increases in indices in the presence of bacteriophage. D'Herelle[2] gives figures as high as forty, Smith[11] above thirty, Weiss and Arnold[14] ten. I have recently worked on this problem and although the indices were lower than those mentioned, they were still above those obtained from vaccines. All of these experiments, however, involved only contact of bacteriophage with bacteria and leucocytes. Attempts to produce sera having high opsonic content by immunization with bacteriophage have not given such encouraging results. The significance of this work, it seems to me, lies in the knowledge that high indices are obtainable. It makes such figures as 2.0 or even 2.5 which are about

the best obtainable with vaccines seem little enough as a basis for a popular and widely used method of treatment.

But if faith in antibodies remains undisturbed by such observations, there exist certain facts that constitute a challenge. Besredka[1] has pretty definitely established the existence of immunity without antibody formation and Gay[5] as well as Freedlander and Toomey[4] have not only confirmed his observations but have indicated the true nature of this immunity by demonstrating the function of the clasmatocyte. That such immunity is confined to the cutaneous tissue is suggested by the work of Hoffstadt and her co-workers[8] who demonstrated that agglutinins and complement fixing antibodies are produced by oral vaccination with typhoid bacilli.

If anything was needed further to disabuse our minds of the idea of the ultimate purposefulness of antibodies, it is furnished by the work of Manwarring[10] and others. As a result of their investigations, we are on the point of conceding that antibodies are not formed by the body but are nothing more than altered antigens and that cells although they may assist in the transformation are not essential to it. True this is in no sense a denial of the existence of antibodies, but by transferring the emphasis we eliminate the feeling of the divine purposefulness of these substances and thus remove what after all has been a very important factor in attempts to connect them with immunity.

It thus appears that vaccination whether for therapeutic or prophylactic purposes is based upon a long vulnerable theory which has in the past few years suffered such buffering as to render it practically useless. Certainly it provides no very firm foundation as a basis for present day practice, and we are faced with the necessity of justifying our procedures by other means, namely practical experience, or of changing the theory. Clinical experience is apparently not giving much comfort; new theories do not promise to develop along lines favorable to vaccination as now practiced. Biologic therapy is progressing towards a substitution of products more closely resembling the living intact but attenuated virus for the vaccine as now used.

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THE USE OF STOCK VACCINES AS A NON-SPECIFIC TREATMENT OF RESPIRATORY INFECTIONS IN CHILDREN*

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Infections of the respiratory tract contribute about three-fourths of the morbidity of childhood, at least in the winter months. Medical science has sought in vain for specific drugs, vaccines, or sera which might prove of value in the reduction of the morbidity and mortality due to respiratory infections. The pediatrician, like the immunologist, has dreamed of that Utopia in which specificity reigns supreme. We have been reluctant to admit that tuberculosis and syphilis cannot be made to yield to a specific treatment just as diphtheria has done. And yet, a glance at mortality and morbidity statistics convinces us that the progress made in the treatment of tuberculosis and syphilis is almost as spectacular as the results achieved in the specific treatment and in the prevention of diphtheria. Sunshine, natural and artificial, mercury, bismuth, arsphenamine, malaria, and typhoid vaccine have all been found to be non-specific agents of unquestioned value. Is it, therefore, unorthodox or heretical to claim for stock mixed respiratory vaccines a certain non-specific value in the treatment of respiratory infections? The actual areas involved in these infections may or may not be of great significance. At any rate, specificity is not to be implied, with the facts now available.

We are probably agreed that the Bordet-Gengou bacillus is the etiologic agent in pertussis. We also admit that an emulsion of Bordet-Gengou bacilli is not effective in preventing whooping cough in exposed cases, but that it has a certain very limited value in the treatment of this disease. This therapeutic value is

dependent upon very large dosage and can probably be duplicated by a vaccine containing no pertussis organisms, according to Herrman[4] and confirmed by my own observations.

We are also agreed that the use of respiratory vaccines for the prevention of colds has not produced results suggestive of specificity, although no less an authority than Kolmer[5] has noted a relative immunity to colds in vaccinated adults. My experience with the stock vaccines used for prophylaxis against colds in a few children has been most disappointing and tends to strengthen my belief that since respiratory infections are not prevented by the use of prophylactic vaccine, whatever benefit is noted following therapeutic usage must be non-specific.

Previous to 1928, I was opposed to the use of stock respiratory vaccines or "cold shots" as commonly termed by the laity. The thought of using stock respiratory vaccines as a therapeutic measure did not occur to me until Dr. Harry L. Baum, an otolaryngologist of my acquaintance, pointed out good results obtained with the vaccine in the treatment of certain subacute and occasionally acute infections of the throat, nose and the nasal accessory sinuses. I was prevailed upon to use a mixed respiratory vaccine in the treatment of similar cases and was agreeably surprised to note excellent results. Most of the children treated during the first year suffered from subacute or chronic rhinopharyngitis associated with antrum infection, and usually exhibited a troublesome bronchial cough. These cases are frequently diagnosed glibly as "broncho-sinusitis," and constitute one of the most difficult problems for either the pediatrician or the otolaryngologist.

Results in this small group of cases were so gratifying that I was encouraged to try the vaccine in acute respiratory infections. An opportunity was soon presented during a seasonal epidemic of so-called influenza in the winter of 1928–1929. The vaccine was used only for such patients as had a relapse accompanied by fever and cough. Relapses in cases of influenza are notoriously frequent and are dreaded more than the initial attack on account of the frequency of complications, especially bronchitis and bronchopneumonia. Careful records were kept in 100 cases and com-

pared with those of 100 control cases of influenza with relapse in which the patient did not receive vaccine. It was found that the vaccine treated patients did not develop chest complications after vaccine was begun, whereas in the control series five children developed either bronchitis or bronchopneumonia while under careful observation and treatment. However, the incidence of other complications, notably otitis and cervical adenitis, was in no way affected by the vaccine therapy. Subsequent experience has repeatedly confirmed this observation, and I never advise the use

TABLE

SUMMARY OF RESULTS OF VACCINE THERAPY IN 250 CHILDREN TREATED DURING AN EIGHTEEN MONTHS PERIOD 1928–1929

Rise in temperature was seldom encountered and not recorded in subacute rhinopharyngitis and subacute bronchitis. The cases of bronchopneumonia were all early cases treated in the home. Results with hospitalized cases of pneumonia are recorded elsewhere.[3]

	NUMBER CASES	AVERAGE AGE	AVERAGE NUMBER INJECTIONS	PERCENTAGE SHOWING TEMP. RISE	PERCENTAGE RESULTS RECORDED AS		
					Good	Fair	Poor
Acute throat infections..........	67		1.28	46.5	62.8	29.8	7.4
Acute rhinopharyngitis..........	39	4.8	1.9	55.0	60.0	20.0	20.0
Subacute rhinopharyngitis.......	13	7.0	3.7		92.3		7.7
Acute laryngitis.................	27	3.0	1.4	78.0	55.5	40.8	3.7
Acute bronchitis.................	60			69.0	80.0	18.3	2.7
Subacute bronchitis.............	8	6.0	3.2		75.0	12.5	12.5
Asthmatic bronchitis............	16	3.1	2.1	44.0	55.5	22.2	22.2
Bronchopneumonia...............	26	3.4	2.1		75.9	24.1	

of vaccine in cases of otitis, mastoiditis, or cervical adenitis except in cases where there is a concomitant cough or bronchitis.

Having convinced myself of the efficacy of vaccine therapy in both upper respiratory and certain acute lower respiratory infections, I did not hesitate to try vaccine therapy for laryngitis also for acute, subacute, and asthmatic bronchitis, and in pneumonia.

It is my impression that vaccine therapy is not indicated for all infections of the upper respiratory tract but only in cases in which cough or nasal discharge is a prominent symptom. Acute, self-limited infections such as tonsillitis or uncomplicated influenza do

not deserve vaccine therapy. Nor do suppurative otitis or acute cervical adenitis yield to it. The most striking results are obtained in cases of acute laryngitis and bronchitis accompanied by fever. A dry, frequent, unproductive cough becomes loose and productive within twenty-four hours after injection and the temperature frequently returns to normal within thirty-six hours. One injection of vaccine is frequently sufficient to terminate an acute infection, but subacute or chronic infections such as subacute rhinopharyngitis and subacute bronchitis usually require from two to four injections at forty-eight hour intervals and with increasing dosage. Febrile reactions are to be anticipated in the acute infections, but are less common and never severe in the subacute or chronic infections. As a corollary to this observation, the dosage of vaccine may be relatively greater in the subacute infections. The tabulation summarizes some of my observations during a period of eighteen months when the vaccine was put on trial and careful observations recorded in each case.

It is desirable to use a heavy bacterial emulsion so that large dosage may be administered in a quantity not exceeding 1 cc. I have been using an emulsion containing 2 billion organisms per cc., the dosage varying from .1 cc. to 1 cc., according to the age and the condition of the patient. Disappointing results may be obtained if an insufficient dosage is administered, especially in severe cases of acute laryngitis or bronchitis where it is hoped that pneumonitis may be aborted by the vaccine. Mild, early cases of bronchopneumonia frequently clear up after one injection. Severe cases require repeated injections and in well advanced pneumonitis the value of the vaccine is questionable. The wheezing and coughing of asthmatic bronchitis are usually promptly relieved by vaccine therapy, but pure asthma not due to infection is not amenable to such treatment.

The criteria for the estimating of results are mentioned elsewhere,[1,2] but it is freely granted that the personal equation greatly influences the interpretation of clinical observations. Having begun this work as a skeptic, it was not difficult to maintain a conservative attitude in the evaluation of results obtained. Surely one does not expect to obtain 100 per cent of good results with a

procedure which is admittedly nonspecific. The average critical physician will not be convinced of the apparent efficacy of vaccine therapy by the results shown in the tabulation. Besides the "personal equation" he finds other unstable components in such a clinical report, such as chance, too small a number of cases, and that ephemeral but powerful factor called by Brenneman the "vis medicatrix naturae."

Far more convincing than tabulated results are two observations which have resulted from these studies; first, that parents have frequently requested the administration of the vaccine for subsequent respiratory infections, and second, that other pediatricians in Denver have begun to use stock respiratory vaccines and found them to be of value.

No discussion of vaccine therapy would be fair or complete without pointing out the disadvantages of such therapy. Such disadvantages are by no means unimportant. The physician does not wish to risk losing the confidence of a child or parent by needle therapy of any kind. It is essential that the smallest sized hypodermic needle be used and that the physician exhibit at once a certain dexterity of both fingers and tongue, talents to be acquired from the prestidigitators of the stage. The local reaction is generally painful for twenty-four hours and not conducive to inviting repeated injections, even though an excellent result is obtained with the first injection. The systemic reaction, sometimes with high temperature for a few hours, is even less apt to inspire confidence in the physician. It is indeed unfortunate that these undesirable local and systemic reactions cannot be avoided. A preparation known as Omniodol containing bacterial products of value for non-specific therapy does not cause severe local or systemic reactions. This product enjoys some popularity in European clinics, but it is expensive and inconvenient to use. I have had insufficient experience with the preparation to say whether it can be substituted for mixed respiratory vaccine. Finally, the physician using vaccines at the present time runs the risk of labeling himself as a faddist, or at least as being out of step with the profession.

In conclusion, my own experience based on the use of stock respiratory vaccines in approximately 1,000 cases leads me to be-

lieve that this form of non-specific therapy has a definite value in the treatment of certain respiratory infections, and that such value generally outweighs the obvious disadvantages.

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THE ASCHHEIM-ZONDEK PREGNANCY TEST

Its Clinical Application in a Series of Cases[*]

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The need of a satisfactory laboratory test for pregnancy has long been recognized, but the great majority of those which have been introduced have failed to satisfy the requirements for such a laboratory procedure. One of the most outstanding developments in the field of laboratory diagnosis within the past few years has been the Aschheim-Zondek reaction for the diagnosis of pregnancy. This test depends for its value on a sound scientific principle, and is comparatively simple to perform.

Smith and Engle,[4] and Zondek and Aschheim,[5] independently and practically simultaneously, found that a hormone of the anterior pituitary gland has the property of stimulating the reproductive system. It is not specific for either sex, and stimulates alike the ovaries of the female, and the testes of the male. This hormone when injected into immature animals causes a premature maturity, with the establishment of the active reproductive function.

Aschheim and Zondek[1, 2] found, with rare exceptions, that in no condition except pregnancy was there an abundance of the anterior pituitary hormone secreted; but in pregnancy large amounts were liberated and excreted in the urine. The test is based on the above facts.

The technic of the test is quite simple, and requires no particular skill either in operation or interpretation. Two points how-

ever must be kept in mind: first, the age of the animals, which in the case of mice must not be less than twenty-one nor more than twenty-five days at the start of the test. When rats are used they must not be less than twenty-eight nor more than thirty-five days of age at the start of the test, second, the full number of injections must be given at regular intervals, and the animals must be killed at a definite time after the first injection, ninety-six to one hundred hours for mice, and one hundred and ten to one hundred and twenty hours for rats.

The first specimen of urine of the morning voided in a clean receptacle after careful cleansing of the parts is satisfactory, although a catheterized specimen is more desirable. The specimen is preserved by the addition of a small amount of toluol, and is kept in an ice box. This prevents the loss of animals due to bacterial contamination of the specimen. The hormone is found to be thermolabile (56°C.), and does not readily pass through a Berkefeld filter. For these reasons urinary specimens cannot be sterilized by heat or filtration. The technic as will be described is modified slightly from that of the original workers, and has proven satisfactory.

Young immature female white rats from twenty-eight to thirty-five days old are used. At least two animals are employed for each specimen of urine. Injections of 1.5 cc. of urine are made subcutaneously three times a day, for three consecutive days. One hundred and twenty hours after the first injection the animals are killed, and the ovaries examined macroscopically. The original technic consists of six injections in young female mice over a period of two days, and the animals are sacrificed ninety-six hours after the first injection.

The presence of enlarged ovaries with numerous blood points constitutes a positive test, and the macroscopic examination is sufficient for practical interpretation. These ovaries when sectioned will show numerous developing corpora lutea, many of which are in the stage of the corpora hemorrhagia. There is also marked congestion of the ovary, and this with the corpora hemorrhagica gives the macroscopic picture as mentioned above. In most cases rupture of the follicle has not taken place. The ovum

is contained within the developing corpora lutea, corpora lutea atretica.

A negative test shows the ovaries unchanged, or slightly enlarged, with no blood points present. Corpora hemorrhagica or corpora lutea are not seen on microscopic examination, but there may be some follicular development.

Frequently, there is present in the urine of women a considerable amount of ovarian hormone. This hormone will cause the enlargement and maturation of the genitalia of the test animals, with the exception of the ovaries. This same enlargement and maturation also occurs when urine which contains anterior pituitary hormone is injected, due to the production of ovarian hormone in the ovaries of the test animal. Thus it is evident that the interpretation of the test must be based on the changes in the ovary, rather than on the changes in the rest of the genitalia.

Any test to be of clinical value must give a positive reaction early in pregnancy, before physical signs are present. Aschheim and Zondek,[2] and others have reported satisfactory results on early cases, and the first workers stated that the hormone is more abundant early in pregnancy than during the latter months.

Practical use of the test has been made on suspected cases in the hospitals and out-patient department of this institution, also on cases referred by physicians. All cases were followed to delivery, or until definitely proven pregnant, or not pregnant clinically. In the series of cases tested, the majority were early pregnancies and non-pregnant conditions suggestive of pregnancy. In addition, a small number of cases of pregnancy in the later months were run. A moderate number of cases not concerned with pregnancy were also tested, in an attempt to find some other condition which might cause the same reaction as pregnancy. A small series of urine specimens from males proved so consistently negative that they were discontinued.

Occasional reactions are encountered which cannot be read as definitely positive or negative. The test is repeated in these cases with a fresh urinary specimen before a final report is made.

The tabulation shows that in this series false positive reactions are the more common. A brief summary of a case in error may be of interest.

Mrs. C., aged thirty-seven years, white, weight two hundred and ninety-six pounds. On March 25, 1930, patient stated she had missed one period. Examination was not satisfactory on this date. An Aschheim-Zondek test was run on a specimen of urine collected at this time and gave a doubtful result. A second test was run on a specimen of urine obtained April 8, 1930. Ovaries of the test animals were normal. Reported negative. May 8, 1930, vaginal examination showed a firm cervix, probably no pregnancy. A third test was

TABLE

RESULTS WITH MODIFIED ASCHHEIM-ZONDEK TEST

	POSITIVE REACTIONS	NEGATIVE REACTIONS	PER CENT ERROR
Cases of pregnancy:			
Less than three months..............	90	1	1.1
Three to six months................	37	1	2.63
Six months to term.................	17	0	0.0
Total............................	144	2	1.37
Non-pregnant cases:			
Males............................	0	34	0.0
Females..........................	4	71	5.33
Total............................	4	105	3.67
Total error in 255 cases...................................			2.35

run on a specimen of urine obtained at this time. Ovaries of the test animals were enlarged and quite hemorrhagic, and were reported as positive. On July 12, 1930, patient entered hospital complaining of bleeding from the vagina, nausea, and pains in lower abdomen and back. There was a history of three pregnancies resulting in abortions. Diagnosis of threatened abortion was made. Bleeding and pains ceased on the second day and patient was discharged on July 17, 1930. October 10, 1930, no evidence of pregnancy could be found on examination and roentgenogram of the abdomen was negative for pregnancy.

This case may have had a missed abortion prior to her hospitalization on July 12, a very likely possibility in view of her previous history.

The following case illustrates very well the early date after conception that the urine may give a positive reaction.

Mrs. M., (Courtesy of Dr. G. C. Main), aged thirty, white. Husband was home over night for first time in six weeks. Patient should have menstruated three days later but did not. Test was run ten days after coitus. Ovaries of the test animals were large and hemorrhagic, a positive reaction. Patient aborted one month after coitus, and the products of conception were shown to her physician who estimated them to be of not more than one month's duration.

The great similarity between the ovaries of young animals injected with urine from a case of pregnancy and the ovaries of young animals which have received anterior pituitary transplants is rather conclusive evidence that the test demonstrates the presence or absence of the anterior pituitary hormone. This hormone is sex nonspecific, since in addition to the ovarian changes seen in young female rats, the immature males will show testicular enlargement and maturity of external genitalia when given anterior pituitary transplants, or injected with urine from a case of pregnancy.[3] These facts substantiate the statement of German workers that the anterior pituitary hormone is the motor hormone of the reproductive system.

Young males may be used for the test, but they are not so satisfactory as the young female animals, since the results are not so clear cut, and the former require a longer time interval to develop the characteristic changes.

SUMMARY

The test has given this laboratory a very satisfactory means for the differential diagnosis of early pregnancy, since the low percentage of error ranks it with the most accurate of laboratory procedures. In many cases very definite and valuable aid has been given the clinician in the diagnosis of the presence or absence of pregnancy at a time when the clinical signs were of little or no help.

The use of rats in place of mice has proven very satisfactory, for several reasons. The rats are larger, mature later, and so can be used when older and better able to withstand infection. The mortality in these test animals is practically nil as compared with

the large number lost when mice are used. It is also much easier to handle the larger animals, and to read the results macroscopically.

Toluol as a preservative of the urinary specimens has proven very satisfactory, since it in no way effects the hormone content of the urine and does keep down the bacterial contamination. By its use specimens may be sent from a considerable distance, and run at a central laboratory. Specimens a week to ten days old preserved with toluol have been tested and have given accurate results.

The results as obtained by the procedure used in this series have failed to show any material difference in the quantity of hormone early or late in pregnancy. They do show that the hormone is demonstrable very early in pregnancy, which makes the test satisfactory as an early diagnostic aid.

Modifications with the use of rabbits offer a shortened time interval, but for routine tests white rats have proven more economical, require less technical skill, and the time element, with rare exceptions, has been negligible.

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RECURRENT AGRANULOCYTOSIS

Report of an Unusual Case

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Agranulocytosis has come to be recognized as a clinical entity that has a definite pathology, as a disease that is dramatic in its onset and course and usually fatal in its outcome. Though the cause of the disease remains unknown at this time, much evidence has accumulated to shed light on the course of events and pathological picture.

The case report in this paper is of unusual interest because of the recurrent acute attacks interspersed with prolonged periods of chronic granulopenia, the possible association with previous administration of typhoid vaccine, and the coincidence of a severe methemoglobinemia. It is also of interest because of the recorded blood counts during these periods, by means of which further evidence is afforded that the onset in the bone marrow precedes the appearance of clinical symptoms.

CASE REPORT*

The patient, white, aged forty-four years, was a housewife with four healthy children, and lived under good economic conditions.

Her history included bilateral salpingitis in 1915, bilateral otitis media following scarlet fever in 1918, with a partial deafness since that time. She had always been subject to frequent attacks of colds and conditions diagnosed as influenza. She has had a large number of courses of various types of vaccine therapy during the previous ten years, including catarrhalis mixed vaccine three times, typhoid vaccine three times, and mixed pneumococcus vaccine twice, each course consisting of from four to eight subcutaneous doses.

For the previous five years she had complained of periods of weakness, which on some occasions had necessitated her giving up her household duties and

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* For the privilege of reporting this case I am indebted to Dr. E. Bates Bloch, Atlanta, Ga.

staying in bed for a few days. No blood counts had been done in those periods. One of her chief complaints was habitual constipation. For five years, she had had a peculiar slate color of skin and mucous membranes, which was later determined to be due to methemoglobinemia. For six months prior to the attacks of agranulocytosis she had been treated for bilateral maxillary sinusitis with little improvement, though the infection was not purulent at any time.

About four weeks prior to the first attack she had received the last of a series of five doses of prophylactic typhoid inoculations, each being followed by a severe reaction, including fever to 103 degrees. She had not been entirely well following the final dose, but had had a mild fever daily for the four weeks following. Because of this, I reported her case before the Fulton County Medical Society, Atlanta, Georgia, as "Agranulocytosis following prophylactic typhoid inoculations," though at this time I am uncertain as to the rôle this played in producing her first attack. Bromberg and Murphy[1] have reported one such instance. However, the effect of typhoid vaccine on the neutrophiles is a disputed question. Cowie and Calhoun[2] state that it is followed by a leukopenia, while Smith[3] states that it is followed by an initial leukocytosis with a mild leukopenia four days later.

I first saw the patient on May 13, 1930. At that time, she had fever of 102 degrees, was in bed, very weak and tired, though she had no pain. Her condition was essentially the same as it had been for the previous four weeks. She was listless and apparently unconcerned with her condition. Physical examination showed a thin, frail, emaciated white woman with a peculiar slate color of the skin and mucous membranes. There were no abnormal findings of the chest or abdomen, nor ulcerations of the mouth or throat.

The white cell count was 900 with no demonstrable neutrophiles. Daily blood counts were begun with the results as shown in the accompanying table. For one week her condition remained about the same, with little apparent change. On May 19, the count fell to a low figure of 470 leukocytes per cu. mm. with no neutrophiles. Her death was predicted during this period. On May 21, she was sent to Emory University Hospital with a white cell count of 930 and 16 per cent neutrophiles. On that date she received a stimulating dose of x-ray to the long bones, with the blood count on the following day being 2600 and 57 per cent neutrophiles. In this instance, I believe the x-ray therapy was of considerable value.

She steadily improved clinically, with less fever daily, was more alert mentally, and received a second dose of x-ray on the 23rd, and a third dose on the 24th of May. On the latter date her red cell count was 4,420,000; on May 26, it had fallen to 1,190,000. This seemed so remarkable that it was rechecked several times with both venous and capillary blood. The red cells then reached a low point of 1,000,000 on May 27. Because of this, she was immediately given a transfusion of 500 cc. of whole blood. In forty-eight hours the count was again found to be more than 5,000,000. I am unable to explain this remarkable

TABLE
Summary of Blood Counts

DATE	LEUKO-CYTES	NEUTRO-PHILES	ERYTHRO-CYTES	HEMO-GLOBIN	REMARKS*
		per cent		per cent	
5-13-30	900	0	5,300,000	95	
5-13-30	930	0			
5-14-30	660	0			
5-15-30	750	0			
5-15-30	900	8			
5-16-30	950	9			
5-16-30	1,000	12			
5-17-30	740	0			
5-18-30	580	0			
5-19-30	470	0			
5-20-30	1,000	24	5,300,000	95	
5-21-30	930	16			Admitted to hospital
5-22-30	2,600	57			X-ray therapy
5-23-30	800	34	4,580,000	90	X-ray therapy
5-23-30	880	36	4,700,000	90	
5-24-30	1,300	50	4,420,000	85	X-ray therapy
5-26-30	1,400	56	1,190,000	55	
5-27-30	1,600	64	1,040,000	50	X-ray therapy
5-27-30	1,400	70	1,000,000	50	Transfusion
5-28-30	1,440	70	2,220,000	70	
5-29-30	2,200	62	5,130,000	85	
5-30-30	2,400	76	4,470,000	80	
5-31-30	2,000	75	4,900,000	90	
6- 2-30	3,150	76	5,110,000	90	
6- 3-30	2,950	80	4,700,000	85	X-ray therapy
6- 4-30	2,400	79	4,440,000	85	Left hospital
6- 6-30	3,250	84	5,270,000	95	
6-10-30	4,150	80	5,350,000	95	
6-12-30	5,100	83	5,500,000	95	
6-16-30	5,000	79	4,900,000	90	
6-18-30	4,550	76	5,000,000	90	
6-20-30	4,650	72	5,250,000	90	
6-26-30	2,650	58	4,900,000	90	
6-27-30	2,600	60	5,100,000	95	
6-28-30	4,200	70	5,400,000	95	Admitted to hospital
6-30-30	2,900	62	4,850,000	90	Pleuritis with effusion
7- 1-30	2,400	65			
7- 2-30	3,500	71			
7- 3-30	3,700	62			
7- 5-30	3,650	68			
7- 6-30	3,150	62			

TABLE—*Concluded*

DATE	LEUKO-CYTES	NEUTRO-PHILES	ERYTHRO-CYTES	HEMO-GLOBIN	REMARKS[*]
		per cent		per cent	
7– 7–30	2,750	52			
7– 8–30	3,450	61			
7– 9–30	2,500	58	5,000,000	95	
7–10–30	3,350	72			
7–12–30	3,600	62			
7–14–30	3,350	60			
7–16–30	4,000	64	3,350,000	85	Left hospital
7–18–30	3,350	58	5,000,000	90	
7–21–30	2,900	68			
7–28–30	4,800	62			
8– 7–30	5,700	75			
8–14–30	5,850	78			
8–21–30	5,200	74			
8–28–30	4,650	69			Feeling well. Carrying on household duties
10– 3–30	3,150	52			
10–10–30	2,700	46			
10–20–30	900	0			Feeling well. Recent tooth extraction
10–21–30	200	0			Collapse
10–22–30	200	0			Admitted to hospital
10–22–30	150	0			Transfusion
10–23–30	510	0			Death this date

[*] All blood cultures negative.   No evidence of infection at any time.

disturbance of the erythrocytes, though excessive dosage of x-ray should be considered.

She steadily improved and was discharged from the hospital on June 4, with a white cell count of 2400 and 79 per cent. neutrophiles.   After her return home it was necessary for her to remain in bed because of weakness.   From June 4 to June 28, attempts were made to check her blood count carefully.   During this period the count varied between 2000 and 4000 with the neutrophiles about 80 per cent.

On June 28, she developed a right hydrothorax and was readmitted to the hospital.   It was interesting that the white count for two days prior to this had fallen from a level of 4500 to about 2500.   She remained in the hospital for three weeks, during which time her right thorax was aspirated and a total of 3500 cc. of clear, amber fluid removed on three occasions.   During this period the white count fluctuated between 2000 and 4000, with the percentage of neutrophiles about normal.   She was discharged on July 16, was weak, but sat up, and felt better than at any time in the previous two months.

The outstanding feature of the course of illness up to this time was the low blood count of 470, followed by recovery.   Rosenthal[4] has stressed the point

that counts below 1000 are likely to prove fatal, basing this observation on his series of five fatalities with counts below that figure and five recoveries with counts above that figure.

A period of two and one-half months elapsed from the date of her discharge from the hospital until her re-admission with the third and fatal attack. During this time she was up, feeling well, carrying on her household duties, and was away one month at the beach on a vacation. Ten days prior to the attack the white cell count was 2700 with 46 per cent. neutrophiles. On October 20, the count was 900 with no neutrophiles, although the was feeling as well as usual. Four days before she had had a tooth extracted. On October 21, she collapsed, became profoundly weak and depressed, and went to bed. She stated that she suffered no discomfort, but that she could hardly raise her head from the pillow. Her white cell count on that date was 200 with no neutrophiles. She was admitted to the hospital on October 22, with the white cell count at 150, received a transfusion, and died on the day following, showing no evidence of infection. Apparently she died from the mere absence of the neutrophiles, though some signs of broncho-pneumonia were present just before death. Despite much effort, permission for a post-mortem examination could not be obtained.

DISCUSSION

The leukocytes fell to 470 in the first attack, followed by recovery, which, so far as I am able to determine, is the lowest recorded leukocyte count followed by even temporary recovery of a patient.

In the third attack the patient's white cell count dropped from a low normal of 3000 to 900 with a complete absence of neutrophiles, with the patient showing no ill effects and feeling as well as usual. This observation is similar to that made by Roberts and Kracke[3] in which it was demonstrated that the blood stream changes occurred before evidence of infection was present.

The question of typhoid prophylaxis as a whole or partial cause of the condition in this patient must be considered.

The sudden falling of the leukocyte count from 4600 to 2600 for two days prior to the attack of pleurisy with effusion is significant, and indicates a causal relationship because of loss of neutrophilic resistance.

The association of the condition with methemoglobinemia may be important, since this patient gave a history of using large amounts of coal tar derivatives during the previous three years.

Since it is known that benzene is a powerful leukocytic depressant, and that coal tar derivatives are for the most part altered benzene rings, the possible association should be considered. In this connection it should be noted that coal tar derivatives have had their widest usage since the World War.

This patient had a tooth extracted four days prior to her final attack. Since this has been the history in many of these patients, this phase of the possible etiology should be investigated.

Since the investigation of the possible etiological factors in this field is a tremendous undertaking, it behooves all who have the opportunity of studying these patients to take very careful and painstaking histories in an effort to find a clue that will lead to the detection of a substance that is responsible for the powerful depression of the bone marrow.

CONCLUSIONS

(1) An unusual case of agranulocytosis is reported, which sheds further light on the sequence of events in this disease.

(2) The co-incidence of agranulocytosis and methemoglobinemia is reported.

(3) It is suggested that the probable etiological factor is a rarely used substance containing benzene or one of its derivatives.

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STANDARD NORMALS AND NORMAL RANGES IN HEMATOLOGY*†

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The routine blood count next to urinalysis is the laboratory examination most frequently used in clinical diagnosis. The results of these examinations have always been reported to the clinician in a form which is often difficult to interpret due to lack of uniformity in the value of the figures used as normal. An effort should be made to make the reports more uniform and provide a more definite method for interpreting the results.

The purpose of this paper is to draw attention to certain figures and recommendations appearing in the chapter on hematology in Approved Laboratory Technic by Kolmer and Boerner, which was prepared under the auspices of the American Society of Clinical Pathologists and which is in press. Unless special attention is drawn to them, their significance and general usefulness may be easily overlooked. I refer particularly to the table of Standard Normals and Normal Ranges recommended for use in reporting blood counts and calculating the various indices. It is hoped that they will meet with general approval and that their use will lead to more uniformity in reporting and interpreting blood counts. A better understanding of them can best be reached by a discussion of their application.

It is to be noted that the figures given under Standard Normals (table 1) do not represent normals in the strict sense. The use of average normals of the various authorities would not seem mathematically or scientifically correct, as the average normal

blood is no more 100 per cent than the average normal child is 100 per cent normal. In a stricter sense 100 per cent should be the highest a normal individual can obtain without becoming abnormal. Furthermore, if the average figures were used, about one-half of normal individuals rather than the exceptional one would have over 100 per cent. It being impossible to furnish figures applicable to all localities the situation was met by giving

TABLE 1

Standard Normals and Normal Range*

EXAMINATION	STANDARD NORMAL (100 per cent)	NORMAL RANGE
Hemoglobin (male)...............	17.3 gms. per 100 cc. of blood	14–18 gms. (80–105 per cent per 100 100 cc. of blood
Hemoglobin (female)............	17.3 gms. per 100 cc. of blood	12–15.5 gms. (70–90 per cent) per 100 cc. of blood
Erythrocytes (male).............	5 million	4.5 to 6 million
Erythrocytes (female)..........	5 million	4 to 5.5 million
Leukocytes........	According to age (see table 2)	4 to 11 thousand
Cell volume (male)	50 cc. per 100 cc. of blood	40 to 50 cc. per 100 m. of blood
Cell volume (female)	50 cc. per 100 cc. of blood	35 to 45 cc. per 100 cc. of blood
Volume per cent (male)	Double the cell volume	80 to 100 per cent
Volume per cent (female)........	Double the cell volume	70 to 90 per cent

* These figures are taken from Approved Laboratory Technic by Kolmer and Boerner, D. Appleton & Co., New York, N. Y., 1931.

two sets of figures namely, "Standard Normals" and "Normal Ranges," the former representing figures to be used as 100 per cent whenever results are reported in per cent and for calculating indices. The figures are high enough in the normal range so that only the exceptional will reach 100 per cent. In most instances round numbers were selected to facilitate the calculation of indices.

Acceptance of these standards does not require any change in methods or opinions regarding normal blood findings. It merely

means the reporting of results according to these standards so that reports from all sources will have the same relative value.

The *Normal Ranges* represent as the name indicates, what might be considered as high and low normal findings. This range may not be applicable to all localities or meet the approval of all hematologists and for that reason it is flexible and subject to change, e.g. if anyone disapproves of them he merely has to substitute his own range in terms of standard normals. Further studies of normal blood will give more exact and additional ranges suitable to different localities, races, environment, and so forth. Whatever range is used, it should be expressed in terms of *Standard Normal* so that reports from various sources will be comparable. A better understanding of the use and value of these normals will be obtained by further discussion of each examination.

HEMOGLOBIN

To be exact, 100 per cent normal should represent the largest amount of hemoglobin possible to exist in 100 cc. of normal blood. This would only be found in individuals having the highest normal number of erythrocytes of the largest normal size. Such a figure not being available an approximate figure is given (17.3 grams) which can be used for this purpose without seriously changing the value of the percentages to which the clinician is accustomed.

At present 100 per cent hemoglobin has many values depending upon the scale used for its determination. To overcome this variation and for the sake of uniformity, it has been recommended that the hemoglobin be reported in grams per 100 cc. of blood. This should be given further encouragement and used more generally. However, many laboratories are reporting in percentage due to the clinicians' wish to have it so reported. In such cases if the standard normal of 17.3 is used as 100 per cent there will be but one percentage scale which will represent the same number of grams no matter where the examination is conducted and would tend to uniformity to the same degree as reporting in grams.

By adopting this standard, no one's opinion regarding the average normal or normal range will have to be changed. It merely means that instead of having each authority giving a new value for

100 per cent, they take their position along the line of the percentage scale of the standard normal. The normal range can be decided by the pathologist or clinician to suit his locality.

CHART I*
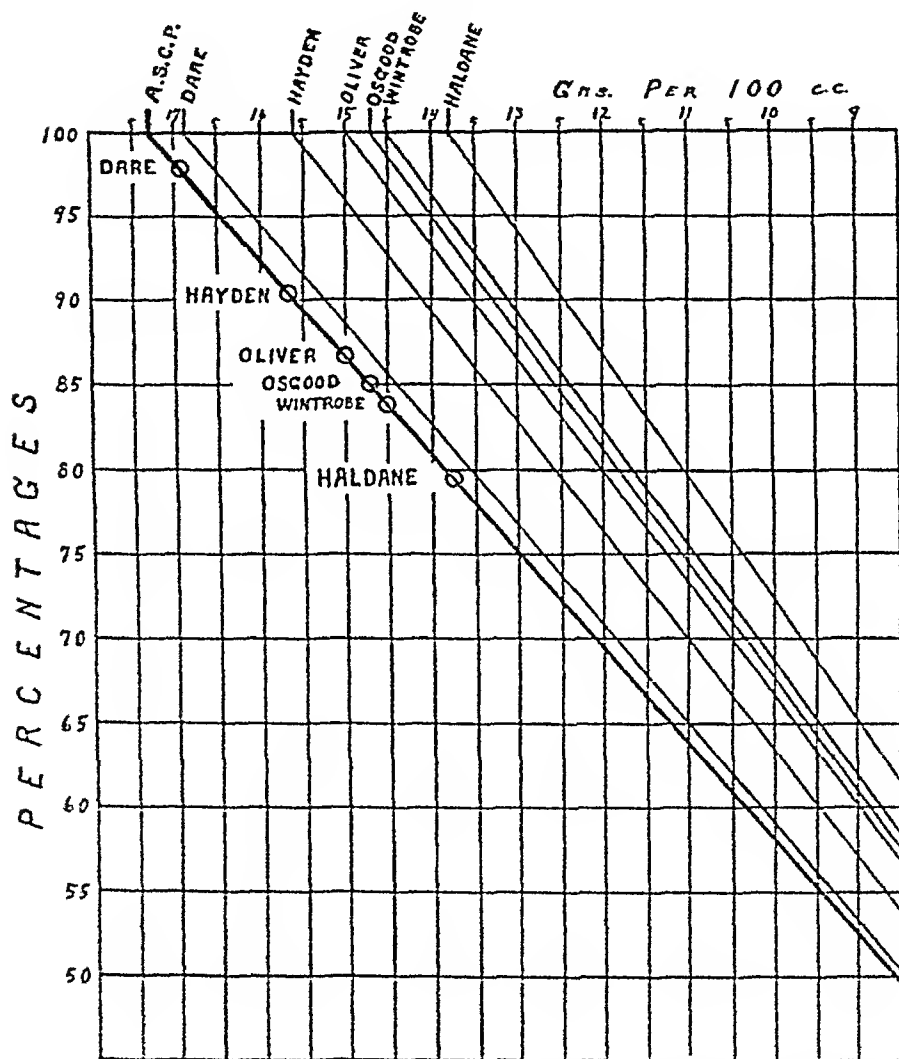
* The Dare standard of 16.9 gms. was taken from Kilduffe's "Clinical Interpretation of Blood Examinations." The standard has been changed several times by the manufacturers of the instrument and now is said by them to be 16.0 gms.

ERYTHROCYTES (NUMBER)

The figures given are those usually employed for calculating indices and are convenient and suitable for this purpose. They are sufficiently high so that a large majority will fall below 100 per cent although more than the exceptional male will be higher. The same figures are given for both male and female although the ranges vary.

ERYTHROCYTES (VOLUME)

Using 50 per cent as 100 per cent normal for packed cells is convenient for calculating indices and the percentage is sufficiently high so that only the exceptional will be higher. Even though the average normal is closer to 40 than 50 the latter is preferred for reasons stated above. To convert the cell volume per cent into per cent of standard normal it is only necessary to multiply by 2.

INDICES

The use of Standard Normals for calculating the color, volume and saturation index will make the readings uniform and of the same value no matter where they are done.

LEUKOCYTE COUNT

Total count. The total leukocyte count should only be used with the differential count for calculating the actual number of each type of cell per cubic millimeter. To use it alone is the same as weighing a patient to determine if there is an increase or decrease in the size of the organs. The range of 4,150 to 11,050 gives the lowest count possible and the latter the highest with all cells within normal range.

Differential count. The differential count should be used first to determine what per cents of the total are neutrophiles, lymphocytes, and so forth, secondly, to study the red cells and note the presence of abnormal cells, and thirdly, to determine if there is a shift to the left of the neutrophiles (table 2).

Reporting counts. The report of counts should be in compliance with the request of the clinician. He desires to know if there is a

decrease or increase in any of the leukocytes. The custom at the present is to report the total number of cells per cubic millimeter of blood with the percentage of the various types. He looks at the total then at the percentages; some think the total most important and others the percentages. As a result there appears to be a consistent inconsistency in interpreting what should be considered normal or abnormal. The method recommended for overcoming this difficulty is to report the findings in total number of each type of cell, e.g. instead of reporting a total of 12,500 cells with 75 per cent neutrophiles. 23 per cent lymphocytes and 2 per cent monocytes, the report would read—Neutrophiles, 9375, lymphocytes, 2875, monocytes, 250 which would be interpreted

TABLE 2

NORMAL RANGE OF LEUKOCYTES

	3 MONTHS TO 3 YEARS	3 TO 5 YEARS	OVER 5 YEARS AND ADULTS
Neutrophiles......	2000–7000	3000–8000	3000–7000
Basophiles......................	0–50	0–50	0–50
Eosinophiles....................	25–700	50–700	50–400
Lymphocytes.........	4000–9000	2500–6000	1000–3000
Monocytes.........	25–700	25–700	100–600

as a slight neutrophilia. It requires less than a minute to make the necessary calculations. The percentages should only be used as a means to an end and not given as a final answer. The following table which is given in Approved Laboratory Technic of the American Society of Clinical Pathologist can be used for interpreting the findings. Like the normal ranges they can be supplemented and further use of them will no doubt furnish more exact ranges and additional ones particularly for children.

Interpreting the leukocyte count. For simplicity and uniformity the following nomenclature is recommended for reporting abnormal findings in the leukocyte count:

    Neutropenia   = decrease in polymorphonuclears.
    Basopenia   = decrease in basophils.
    Eosinopenia   = decrease in eosinophils.
    Lymphopenia   = decrease in lymphocytes.

Monopenia   = decrease in monocytes.
Neutrophilia   = increase in polymorphonuclears.
Basophilia   = increase in basophils.
Eosinophilia   = increase in eosinophils.
Lymphocytosis = increase in lymphocytes.
Monocytosis   = increase in monocytes.

The terms relative and absolute have been omitted as the above terms cover every possible change. A high percentage of

TABLE 3

COMPARATIVE LEUKOCYTE COUNTS USING THE PERCENTAGE AND NUMBER METHOD OF REPORTING

	AUGUST			SEPTEMBER									
	28	29	30	1	2	2	5	6	7	8	9	10	11
Total leukocytes...	3400	2800	1500	450	1500	1100	1400	1300	1000	600	900	450	750
Per cent													
Neutrophiles.......	34	14	29	30	24	18	16	22	12	4	4	20	16
Basophiles.........													
Eosinophiles.......	2	2	1		4	2	4						
Monocytes.........	5	2	1	1				2			8		
Lymphocytes.......	59	82	68	69	72	54	80	76	88	96	88	80	84
Numbers													
Neutrophiles.......	1156	392	435	135	360	198	224	286	120	24	36	90	120
Basophiles.........													
Eosinophiles.......	68	56	15		60	72	140						
Monocytes.........	170	56	15	5				26			72		
Lymphocytes.......	2006	2296	1020	310	1080	880	1120	988	880	576	792	360	630

lymphocytes due to a neutropenia is called a neutropenia and not a relative lymphocytosis, because in the former we state what actually exists and do not involve a type of cell which has not changed from the normal. If the increase percentage is due to an actual increase of lymphocytes, then it is called a lymphocytosis. These terms can be qualified as mild, slight, moderate, marked, and so forth.

To demonstrate further the use and practical value of this method for reporting I would like to present the results of blood

studies in a patient who developed a marked and progressive neutropenia at the Graduate Hospital (table 3).

SUMMARY

By bringing to the attention of the Society the value and purpose of these normal figures, it is hoped that an interest will be stimulated which will lead to their adoption by the members and that measures will be taken to encourage their general use. In conclusion the important points are summarized as follows:

1. The standard normals are not strictly normal, but are figures to be used as standards for reporting blood tests and calculating indices.

2. The normal ranges are what may be considered normal according to standard normal figures. These can be changed to suit the locality or pathologist and are not to be considered standard.

3. The adoption of the standard normal will make all reports of the same value and does not require any change in methods or apparatus.

4. It does not require any change in what was formerly considered normal, except that they will be expressed in terms of standard normal figures.

5. While the pathologists are studying and changing normal ranges, the clinicians will receive reports in uniform values.

6. The use of standard normals does not in any way effect the accuracy of the reports.

7. The interpretation of the reports has to do with the ranges adopted and these can be adjusted to suit the pathologist.

A NEW MODIFICATION OF MALLORY-HEIDENHAIN'S DIFFERENTIAL STAINING METHOD AND ADAPTATION OF FORMALIN-FIXED MATERIAL FOR MALLORY'S STAINS*

JAMES W. KERNOHAN

Section on Pathologic Anatomy, The Mayo Clinic, Rochester, Minnesota

Pathologists are constantly endeavoring to increase their armamentarium to demonstrate better the morbid changes which occur in the tissues of the body. To the histologist, Mallory's stains are invaluable but their uses have been necessarily limited, because tissue to be stained by these methods must be fixed in Zenker's solution. Formalin has become almost the universal fixative because tissue may be preserved in it indefinitely and most stains used as a routine can still be employed. Ever since Mallory described these special methods for Zenker-fixed tissue, histologists have been modifying the fixative and the stains with varying, and usually with unsatisfactory, results. Even washing the tissue and refixing in Zenker's solution is unsatisfactory. Davidoff has suggested placing the formalin-fixed tissue in ammonia water and refixing in Zenker's solution, a procedure which removes some of the difficulties. However, the following adaptation has proved the most satisfactory and uniform method which I have encountered. The formalin-fixed tissues are washed in running water or ammonia water for a short time. The latter procedure serves to prevent the accumulation of "formalin precipitate" on the stained preparation. The tissue is then placed for four days in Weigert's primary mordant for myelin sheaths†

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† Weigert's primary mordant for myelin sheaths:

Potassium bichromate	5 gm.
Chromium fluoride	2 gm.
Water	100 cc.

and for two days in Weigert's secondary mordant for myelin
sheaths.[*]   It is then embedded in paraffin and stained in the
usual way with Mallory's phosphotungstic acid hematoxylin or
other staining methods requiring Zenker's fixation.   This differ-
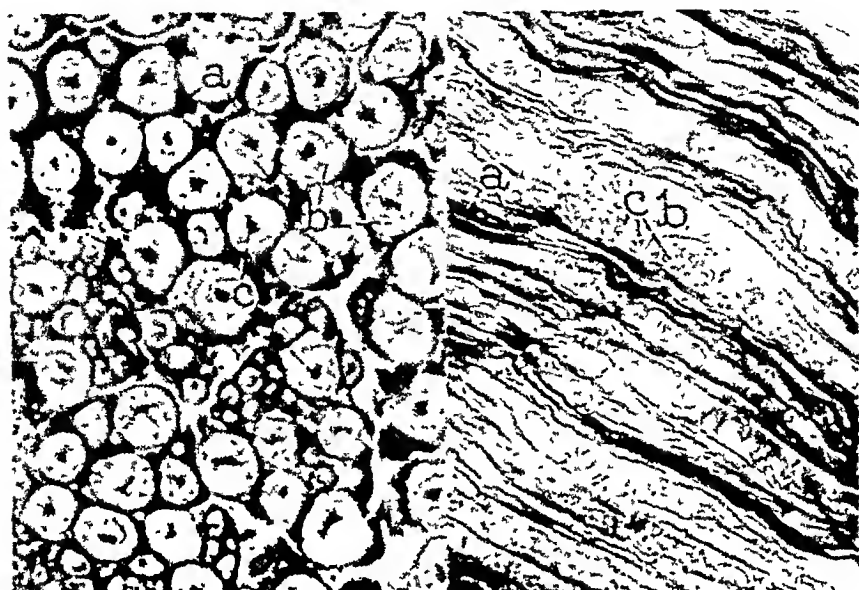
FIG. 1. SECTIONS OF PERIPHERAL NERVES

    The left half of the figure is a cross-section of a normal sciatic nerve; the nerve
was first fixed in formalin and then mordanted with Weigert's myelin sheath
mordants, the connective tissue, a, is a pale blue and the myelin sheaths, b,
are red or deep orange; the axis-cylinders, c, are deep blue or purple (Mallory-
Heidenhain's stain X500).   The right half is a longitudinal section of a degen-
erating femoral nerve; the staining method is the same as in the left section.
The connective tissue, a, is pale blue, the myelin, b, which is breaking down and
collecting in masses, is red or deep orange; in the midst of some of the myelin
masses are small black clots, c, which represent fragments of axis-cylinders which
stain deep blue or purple (X250).

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[*] Weigert's secondary mordant for myelin sheaths:
Acetate of copper	.	.	5 gm.
Chromium fluoride		.	2.5 gm.
Acetic acid, 36 per cent			5 cc.
Water	.	.	100 cc.
Formalin		. .	10 cc.

entiates the neuroglia, fibroglia, and so forth, as satisfactorily and clearly as if the tissue had been fixed in Zenker's solution, and excellent results have been obtained after the tissue has been fixed several years in formalin. Heidenhain's[1] modification of Mallory's ethyl violet orange G. stain is valuable after Zenker's fixation, and the foregoing modification makes it available after
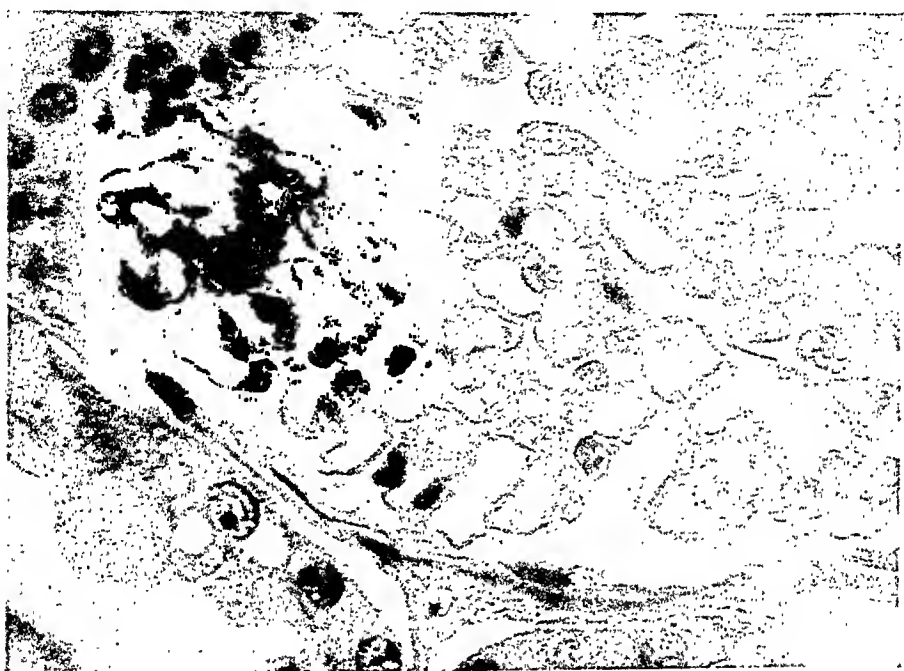
FIG. 2. SECTION THROUGH A GLOMERULUS SHOWING BOWMAN'S CAPSULE AND THE CAPILLARY TUFTS

The connective tissue of the capillaries stains blue and is rendered very prominent with this stain. The basement membrane of the tubules also stains distinctly (Mallory-Heidenhain's stain after fixation in formalin and Weigert's myelin sheath mordants ×720). (After Wilbur.)

fixation in formalin. It is particularly applicable to the peripheral and sympathetic nervous systems. After Weigert's mordant, Weigert's myelin sheath stain may be used, but I have found the Mallory-Heidenhain stain even more valuable, particularly for those nerves in which only slight amounts of myelin are present and from which the hematoxylin so frequently disappears during differentiation. With this stain, the myelin is reddish-orange,

the connective tissue is bright blue and the axis cylinders stain
deep blue or purple (fig. 1). Occasionally, in degenerating nerves
the axis cylinders are deep red, simulating the reverse reaction
of Alzheimer; however, it is not constant. Differentiation is less
drastic than with Weigert's myelin sheath stains and consequently
smaller amounts of myelin retain the stain and are thus rendered
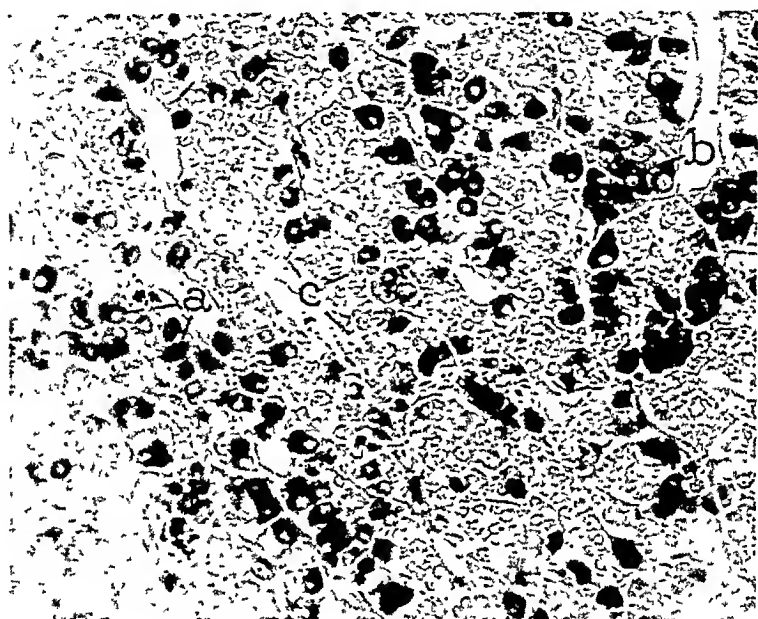
FIG. 3. NORMAL PITUITARY GLAND

With the Mallory-Heidenhain staining method the connective tissue stroma
around the blood sinus and so forth, is pale blue. The chromophil cells, *a*,
are bright red to orange, depending on the length of time the tissue was in for-
malin before being cromated. The basophilic cells, *b*, are deep blue or purple,
depending on the number of chromophil granules present. The chromophobe
cells, *c*, are a pale blue without granules (Mallory-Heidenhain's stain ×300).

visible. This is particularly true in studies of the sympathetic
nervous system. The method has a wide application in the field
of general pathology and histology and in studies on the glomeruli
of the kidney (Heidenhain,[1] McGregor,[2] and others) when differ-
entiation of tissues is important; Wilbur[3] has also found it valu-
able in this connection (fig. 2). It demonstrates the reticulum

of the liver, spleen, lymph nodes, and so forth, and also the blood vessels in hemangio-endotheliomas and other tumors.

Histologists have endeavored for years to differentiate by stains the three types of cells in the pituitary gland, especially by the use of one stain. After fixation in formalin or with the use of unfixed tissue, when mordanted in Weigert's myelin sheath mordants and Mallory-Heidenhain's or azocarmine stain is used, the three types of cells are clearly contrasted (fig. 3). The chromophil cells are bright red, the chromophobe cells are pale blue and the basophil cells are deep blue or purple and readily distinguished from each other. Many cells contain both chromophil and basophil granules, giving the cell a purple tint so that many gradations between the red and blue are visible. The stain is also valuable in distinguishing the chromophil from the chromophobe adenomas of the pituitary gland and shows that many tumors are mixed, containing both types of granules and cells. Bailie'sstain for the granules in the pituitary cells can also be used afterthese mordants.

COMMENT

A method is described which allows the use of Mallory's differential stains on tissue fixed in 10 per cent formalin. This method is applicable to histology in general but to neuropathology in particular. It also differentiates the three types of cells in the pituitary gland.

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(2) McGREGOR, LEONE: The finer histology of normal glomerulus. Am. Jour. Path. **5**: 545–558, 1929.

(3) WILBUR, D. L.: Histologic considerations of the normal renal glomerulus of man. Proc. Staff Meetings of Mayo Clin. **6**: 124. 1931.

EDITORIAL

A Broader View of Comparative Pathology

Perhaps no other group than laboratory pathologists realizes so keenly the changing concept of cause and effect. One of the principal tenets of medical faith was that there was a uniformity of cause and effect to which those in laboratory medicine have too strictly adhered in practicing their mechanistic philosophy. This theory assumed the identity of morbid causes and the entire similarity of anatomical responses. Such a reasoning begins with the history of medical thought.

It is probable that those keen observers, the internists, who, incidentally, often blaze the trail for us, realized very early that there could not be the same response coming from identic causes by every individual. It is a commentary that bears no extension upon the craft of clinical pathologists that too small a number have admitted this and made their calling flexible.

Perhaps the most conspicuous self-analysis that has ever taken place in this country occurred when the Society recently carried out a critical program on specific therapy. If there be one lesson to be learned from these deliberations, it is that there is a missing link in our understanding and practice of what can and should happen in the vaccine handling of certain cases.

The ordinary acceptance of the term comparative pathology implies finding similar morbid changes in snakes, elephants and men. That such a definition is inadequate is illustrated by many observations. Strychnine does not act the same in birds, dogs and man. The amoebic dysenteries, of spontaneous and experimental character, differ in the principal zoological orders. The incidence of tuberculosis in closely related animals is entirely different. Cerebrospinal nerve tract disease does not occur in lower animals. Anatomical structure, and therefore physiology, may vary in different members of the same genus. Certain

infectious diseases occur only in certain genera and may not be transmissible to another. Species specificity of parasitism is well known.

These and many other examples impel us to wonder if we should think of such pathology as contrastive, not comparative.

Comparative pathology should then be limited to intraspecial occurrences, which would depend upon the facts that perfect, common and mutual anatomy and physiology should exist in every member of the species.

Assuming this to be true, one could with accuracy establish certain tests that would have an absolute or mechanistic value. But, with apologies to Claude Bernard, there is a phase of human pathology that must be described on a constitutional basis, there being no such perfect intraspecial uniformity.

One of the hopes of those who practice laboratory medicine cannot fail to be to establish definite measuring rods for constitutional groups in a comparative sense. The reaction to vaccines in the blood tissue atrophies that may be associated with dermatological response, such as psoriasis, scleroderma and the like, cannot be the same as in the case of a plethoric individual with cardiorenal disease. The blood urea nitrogen output cannot be placed on the same basis in sympatheticatonic persons, as in the stoical manual laborer. The pigmented individual cannot have the same pigment chemistry, and therefore the same protein chemistry, as the blond who has the susceptibility to blood diseases.

The goal then towards which to work in comparative pathology is the establishment of standards closely comparable to clinical standards until clinical material has been so tabulated by mathematical measurements that by a simple formula the value of each one of the variables may be transferred into the value for the other. To what extent the field of laboratory medicine is expected to make diagnoses, and therefore to use pure mechanistic rules, cannot be estimated. But if he who practices it will understand that his experimental lower animals are more likely to present contrastive phenomena and his intra-human examples better considered as giving comparative phenomena, and if he

will realize that each of the individual groups is really sui generis, then will he begin to establish his measuring rods and to contribute to the ever deepening significance of racial variation, the parts of which at the moment fail to stand in mathematical relationship.

This cannot lead to the practice of vitalistic philosophy but it forces greater attention to the species norms, finer divisions into groups, closer study of individual cases, and better analysis of comparative pathology within the intra-human responses.

HERBERT FOX.
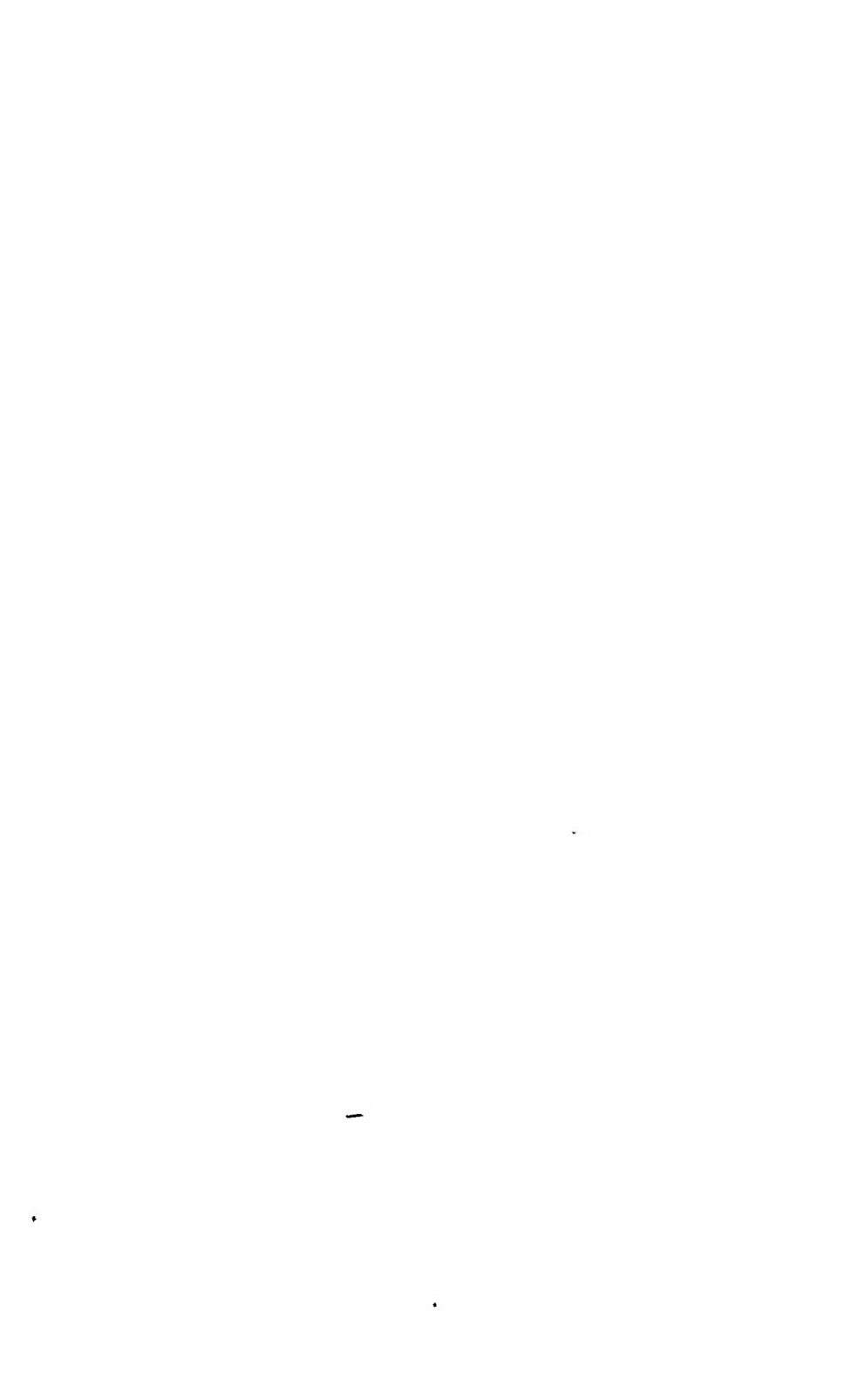
SOCIETY NEWS AND NOTICES

TENTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

June 7 to 9, 1931, Philadelphia, Pennsylvania

THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS held its Tenth Annual Convention at Philadelphia, June 7–9, 1931. The meeting was one of the most successful ever held, about 100 members registering, and demonstrated that the Society has really become of age.

There were twenty-six papers presented during the scientific sessions and there were ten exhibits. The sessions were well attended, there being as many as 200 in the room at times. An interesting and important feature of the meeting was a symposium of eight papers on vaccine therapy.

The annual banquet was an unusual success and was well attended. The three addresses were delivered by President Kenneth M. Lynch, Dr. B. C. Crowell and Dr. David Riesman. These addresses complimented and supplemented each other in an interesting manner and defined well the field of clinical pathology and its relation to medical sciences. Its importance was clearly pointed out and the qualifications of men practicing the specialty placed on a high plane.

The scientific sessions were held, quite fittingly, in the auditorium of the Philadelphia General Hospital. From the windows of this beautiful structure one could almost see the little old brick building in which Osler did his autopsies and in which many members of the Society obtained their fundamental training in the subject. On view for the members were Dr. Osler's old autopsy table and the original protocols of his examinations. The academic and scientific atmosphere of the building added to the spirit of the meeting.

Important changes in the By-laws were passed which will be found elsewhere in the JOURNAL. The following men were elected to membership: Samuel T. Lindsay, Rochester, N. Y., Roy R. Kracke, Emory University, Georgia, Henry F. Hunt, Danville, Pennsylvania, Marcos Fernan-Nunez, Milwaukee, Wisconsin, Plinn F. Morse, Detroit, Michigan, Hazel R. Prentice, Kalamazoo, Michigan, Frederick O. Zillessen, Fond du Lac, Wisconsin, Benjamin E. Konwaller, Davenport, Iowa, William A. Meyers, Oklahoma City, Oklahoma, and Eric A. Fennel, Honolulu, Hawaii.

The Business meeting was held on Tuesday evening June 9. The following reports were presented and approved:

REPORT OF THE EXECUTIVE COMMITTEE

The Committee wishes to report that the year just closing has been a highly successful one. The affairs of the Association have gone forward in a most satisfactory manner.

The work of the Secretary's office has been carried on efficiently by Dr. Giordano and the Chairman wishes to express his appreciation of the pleasure he has had in working with him. The books of the Secretary have been checked and found in perfect order.

The audit of the accountant shows the financial condition of the Association to be excellent. The assets show a slight increase in spite of the launching of the official JOURNAL. Each year has shown a greater financial strength and, though our resources are still small, our condition is sound.

The Committee feels that the Society may be justly proud of the JOURNAL. The material and the mechanical features of the periodical have secured universal approbation. That the Society is able to publish such a JOURNAL with no change in the dues from members is a cause for congratulation. To the Editor and to the Publication Committee we owe a debt of gratitude.

The change in time of meeting was agreed upon as an experiment this year. An expression from the membership as to its continuance is desirable.

A number of changes in the By-Laws have been proposed by this Committee. We trust they will have your thoughtful consideration.

The Committee feels that we have had unusually efficient service from the various committees and wishes to thank them for their labors.

J. H. BLACK,
Chairman.

REPORT OF THE EDITOR-IN-CHIEF

Due largely to the untiring efforts of Dr. J. A. Kolmer, a contract was entered into with the Williams & Wilkins Company for the publication of the

AMERICAN JOURNAL OF CLINICAL PATHOLOGY. It was decided by the Publication Committee that the JOURNAL should be a bi-monthly publication, beginning with January 1, 1931. Three issues have been published and the fourth is in press.

The response on the part of the membership has been most gratifying. The makers and dealers of scientific equipment have contributed advertising most generously, and the JOURNAL has begun under very favorable conditions.

Certain rules have been published in the JOURNAL governing the type of material to be published and the manner in which it will appear.

The Editor wishes to thank the Society for its coöperation and to request its future support for the JOURNAL.

<div style="text-align: right">

T. B. MAGATH,
Editor.

</div>

THE COMMITTEE ON EXHIBITS

Your Committee on Exhibits begs to express the opinion that no exhibit at this meeting showed a degree of excellence sufficient to warrant the bestowal of a medal on the exhibitor.

It wishes, however, to commend Dr. B. Steinberg for his excellent illustrations on The Pathology and Etiology of Degeneration and Inflammatory Lesions of the Appendix and the Presbyterian Hospital of Philadelphia for the beautiful examples of photography in color.

<div style="text-align: right">

W. S. THOMAS,
Chairman.

</div>

REPORT OF SERVICE BUREAU

The activities of the Service Bureau for the year 1930–31 have continued as in the past. Four members placed their names on registration and six institutions consulted this Bureau for service.

Your committee, in order to be effective in its service, urges that every member who would consider change in location to place their name on file with this committee so that they can be immediately notified as soon as an opening is available. While notices of such openings will be published in the JOURNAL obviously very often too much time elapses from the time of the call to the publication of the notice.

<div style="text-align: right">

A. S. GIORDANO,
Chairman.

</div>

REPORT OF THE RESEARCH COMITTEE

In the absence of the Chairman, Dr. A. G. Foord, Dr. M. Pinson Neal, Acting Chairman, reported that the activities of this Committee have been rather limited because of the failure of most of the members to add their personal cases of blood dyscrasias to the Society's permanent registry. It was stated that only four members contributed case records and slides so far and

it is hoped that many more will be contributing in the future. The subjects of agranulocytosis, acute leukemia, blood dyscrasias following arsphenamine therapy and pernicious anemia like syndromes were chosen because of universal interest in these conditions and because of the ease of collection.

The Committee recommended the Huppert-Nakayama test for the presence of bilirubin in urine, based upon favorable reports received.

The Committee recommended that the hematologic collection be continued for the next few years and is also desirous of receiving comments for future studies.

The Committee again made a plea to the Executive Committee to set up more definite rules for making the annual Ward Burdick award. The award this year was granted to Dr. W. G. Exton.

M. PINSON NEAL,
Acting Chairman.

REPORT OF THE PUBLICATION COMMITTEE

The Publication Committee reported that the book on Approved Laboratory Technique, prepared under the auspices of the Society, is finished and is expected to appear from the press in August.

J. A. KOLMER,
Chairman.

REPORT OF THE BOARD OF REGISTRY

The Board of Registry submits a very extensive report of their activities and also submits a financial statement which is referred to the Executive Committee for approval.

They have approved 6 universities and colleges that give courses in medical technology, 5 universities hospital laboratories, 14 hospital laboratories and 6 hospital laboratories conditionally.

Attached to this report is a very extensive investigation by Dr. K. Ikeda pertaining to institutions available for training of technicians and medical technologists.

P. HILLKOWITZ
Chairman.

REPORT OF THE SECRETARY-TREASURER

The year of 1930-31 marks an important step in the history of the American Society of Clinical Pathologists—namely, the publication of the official JOURNAL and the completion of the Book on approved laboratory methods sponsored by your Society. For these accomplishments the Society owes a debt of gratitude to the untiring efforts of Dr. John A. Kolmer for bringing about the arrangements with the publishers; to Dr. T. B. Magath as Editor-in-Chief of the JOURNAL belongs the credit for the high standard upon which the JOURNAL

is run. This office carries many responsibilities and there is a great deal of labor in editing material submitted for publication.

The publication of the JOURNAL without increasing the membership dues has been made possible by greatly curtailing office expenses. For the present the Secretary's office is conducted with only a part time stenographic service and so far the rental of space has been donated.

For the good of the Society it is imperative that we create a reserve fund for future development of our activities. This can be accomplished only by coöperation of our entire membership in eliminating all unnecessary correspondence. This year we have requested that members make their own reservations with Hotels. Since the publication of our JOURNAL, circular letters have been reduced to a minimum. I, therefore, urge you to read carefully the column on Society news and notices and hope that every member contribute to this by writing directly to the Editor-in-Chief.

Since your Society is obligated to pay $5.00 of its dues for the JOURNAL, it is obvious that no member should expect to receive the JOURNAL without meeting his financial obligation. If you are not receiving the JOURNAL or have changed address, please notify your Secretary's office at once.

There are at present 376 active members. During the year 2 members resigned because of ill health and 4 were lost to the Society because of death.

The Secretary wishes to thank the Committee chairmen for their splendid work and coöperation. All the committees have functioned independently and due credit should be given them for their accomplishments. This is particularly ture of the Committee on Local Arrangements. They have done their work efficiently and deserve thanks from our membership.

The following financial report, as approved by the Executive Committee, shows the standing of the Society at the close of the fiscal year, May 31, 1931:

STATEMENT OF RECEIPTS AND DISBURSEMENTS FROM JUNE 1, 1930 TO MAY 19, 1931

Balance in bank, May 31, 1930 (Goldberg, Fox & Co. Report).... $3,371.40
Receipts:

Initiation Fees	$425.00
Membership Dues	3,410.00
Technicians Registry	1,530.28
Convention Expense-Refund	484.96
Commercial Exhibits	180.00
Lantern Sold	30.00
Telephone Refund	2.19
Miscellaneous	3.50
Collections on Checks returned by banks	31.75
Total receipts	6,097.68
Total cash to be accounted for	$9,469.08

Disbursements:

Initiation Fees Refunded		$195.00
Membership Dues Refunded		10.00
Technicians Registry		1,034.50
Convention Expense		1,209.39
Salary—Marie Boes	$195.00	
—R. H. Broderick	100.00	
—Alice Gracy	250.00	
—Stenographic Service	19.25	564.25
Office Rent		25.00
Office Supplies		24.95
Telephone		6.82
Postage		81.80
Printing and Multigraphing		332.91
Goldberg, Fox & Co. Audit		37.50
Freight and Cartage		111.34
Miscellaneous		26.29
Williams & Wilkins Subscriptions		1,780.00
Uncollected Checks returned by Banks		41.75
Total disbursements		$5,481.50
Balance in bank, May 19, 1931		$3,987.58

REPORT OF THE NECROLOGY COMMITTEE

In Memoriam

Take them, O Father, in immortal trust!
Ashes to ashes, dust to kindred dust,
Till the last angel rolls the stone away,
And a new morning brings eternal day!

Oliver Wendell Holmes, M.D.

During the past year the American Society of Clinical Pathologists has lost through death four of its members.

We wish to express to their families our sincere sympathy and our appreciation of the valuable services our late colleagues rendered to the public and medical profession in their efforts in the field of clinical pathology.

Edward Fenton Cooke, Houston, Texas. Born in Oldham, England, August 24, 1875. Common and private school education leading to Fort Worth School of Medicine where he graduated in 1897. Licensed under the Act of 1907 in Texas. He practiced at Houston, then at Forreston, Texas, and then returned to Houston where he has been since 1911.

Died January 8, 1931, age 55, of chronic myocarditis.

Dr. Cooke was a member of the American Medical Association, Society of American Bacteriologists, South Texas Medical Association. He was secretary and treasurer of South Texas Medical Association for six years and in October of 1913 was chosen as President of that Association. He was an active member in the Texas Society of Clinical Pathologists and in the affairs of the American Society of Clinical Pathologists.

Frank Riecker Lyne, Chagrin Falls, Ohio. Born in Stockport, Ohio. Graduated Lowell, Ohio, High School, 1914. A.B. Marietta College, 1917. Graduated Western Reserve University School of Medicine, 1922. Licensed in Ohio, 1922.

Died in Chagrin Falls, June 19, 1930 of a bullet wound.

Dr. Lyne was a member of the Ohio State Medical Association and member of the American Medical Association, Society American Bacteriologists; was on staff of Fairview Park Hospital, Cleveland, Ohio, as pathologist. He was the son of Dr. George L. Lyne of Medina, former County Health Commissioner.

Harold Gustavus Palmer, Philadelphia, Pa. Born in Chicopee, Mass., November 5, 1873. Graduated from University of Pennsylvania School of Medicine in 1895. Licensed in Philadelphia 1896 and in Pennsylvania 1926. Practised in Pittsburgh, Pa., and Providence, R..I. 1915–1918. Was on the staff of St. Elizabeth's Hospital, Washington, D. C., in 1924; St. Agnes Hospital, Philadelphia at his demise.

Died in Philadelphia, American Oncologic Hospital, March 15, 1930, bronchopneumonia. Age 57 years.

Fellow of the American Medical Association and Rhode Island Medical Society.

James Roe Snyder, Sacramento, California. Born in Scriba, New York, November 2, 1888. Graduated from Chicoa, California, High School. Graduated from Syracuse University College of Medicine 1912.

Fellow of American Medical Association; was in City Laboratory in Sacramento, California at time of his death.

<div align="right">J. J. MOORE,
Chairman.</div>

The Nominating Committee presented the following names:

President-elect: WALTER M. SIMPSON
Vice-President: C. J. BUCHER
Executive Committee: K. M. LYNCH
     A. J. FOORD
Board of Censors: NATHAN ROSENTHAL
     F. M. JOHNS
Board of Registry of Technicians: W. E. KING
     ASHER YAGUDA

The above candidates were unanimously elected. The members retiring from the committees are:

Executive Committee: J. A. KOLMER
     A. H. SANFORD
Board of Censors: ERNST SCOTT
     B. W. RHAMY
Board of Registry of Technicians: A. G. FOORD
     C. Y. WHITE

PAST PRESIDENTS

1922–3	Dr. Philip Hillkowitz	Denver, Colorado
1923–4	Dr. Wm. Carpenter MacCarty	Rochester, Minnesota
1924–5	Dr. John A. Kolmer	Philadelphia, Pa.
1925–6	Dr. Frederic E. Sondern	New York, N. Y.
1926–7	Dr. Wm. G. Exton	Newark, New Jersey
1927–8	Dr. A. H. Sanford	Rochester, Minnesota
1928–9	Dr. F. W. Hartman	Detroit, Michigan
1929–30	Dr. J. H. Black	Dallas, Texas
1930–1	Dr. K. M. Lynch	Charleston, S. C.

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GEOGRAPHIC DISTRIBUTION

* Associate Members.   § Corresponding Members.
† Counsellors.   ** Honorary Members.

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DEADMAN, WM. JAMES ... General Hospital, Hamilton, Ontario, Canada
DE LEON, WALFRIDO ... Kansas Avenue 609, Manila, Philippine Islands
DYKE, S. C ... 9 George St., Wolverhampton, England
§FENNEL, ERIC A. ... The Clinic, Honolulu, Hawaii
FINKELSTEIN, MANLY. ... 1155 Wolseley Ave., Winnipeg, Canada
*GARPER, C. Z ... Peking Union Medical College, Peking, China

KELLY, FRANK L..............U. S. N., First Brigade U. S. Marines, Cape Haitien. Republic of Haiti
NICHOLSON, DANIEL..........Medical College, Winnipeg, Canada

ALABAMA

GRAHAM, G. S................2620 Madison Ave., Birmingham, Ala.
JONES, WALTER C............Tenn. Coal, Iron & R. R. Hosp., Fairfield, Ala.

ARKANSAS

†LEE, D. C..................Leo N. Levy Memorial Hospital, Hot Springs, Ark.

CALIFORNIA

ANDREWS, V. L..............Hollywood Hospital, Hollywood, Calif.
BETTIN, M. E...............727 West 7th St., Los Angeles, California
BOGEN, EMIL................Olive View Sanitarium, Olive View, Calif.
BOLIN, ZERA E..............University of California Hosp., San Francisco, Calif.
BONYNGE, C. W.............2007 Wilshire Blvd., Los Angeles, Calif.
BUTKA, H. E................312 N. Boyle Ave., Los Angeles. Calif.
CUMMINS, W. T.............Southern Pacific Gen. Hospital, San Francisco, Calif.
FOORD, ALVIN G............Pasadena Hospital, Pasadena, California
GLENN, ROBERT A...........Samuel Merritt Hospital, Oakland, Calif.
HAMMACK, ROY W...........523 W. 6th St., Los Angeles, Calif.
HOLLIGER, CHARLES D.......203 Medico-Dental Bldg., Stockton, Calif.
HYLAND, C. M..............Children's Hospital, Los Angeles, Calif.
MARQUEZ, H. G.............Flood Bldg., San Francisco, Calif.
MOORE, GERTRUDE...........2404 Broadway, Oakland, Calif.
O'REILLY, B. C. N.........Flood Bldg., San Francisco, Calif.
†PICKARD, RAWSON J........805 Watts Bldg., San Diego, Calif.
PULFORD, D. SCHUYLER......650 3rd St., Woodland. Calif.
RUEDIGER, E. HENRY........Mercy Hospital. San Diego, California
SHACKFORD, BARTLETT C.....1208 Security Bldg., Long Beach, Calif.
STOWE, W. PARKER..........St. Luke's Hospital, San Francisco, Calif.
THOMPSON, HAROLD A........Electric Bldg., San Diego, Calif.
VICTORS, ERNST A..........Medico-Dental Bldg., San Francisco, Calif.

COLORADO

BLACK, WILLIAM C..........University of Colorado, Denver, Colo.
CARSON, P. C..............557 So. University, Denver, Colo.
CORPER, H. J..............National Jewish Hospital, Denver, Colo.
DOWNING, E. D.............Woodman Sanatorium. Woodman, Colo.
DUNLOP, JOSEPHINE N.......Corwin Hospital, Pueblo, Colo.
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Dr. Kenneth M. Lynch, Professor of Pathology at the Medical College of the State of South Carolina, received the honorary degree of Doctor of Laws from the University of South Carolina at the commencement exercises of the University on June 10.

Wanted: A pathologist in the Department of Pathology of a southern Medical School. Maximum salary $4500.00. Communicate with Secretary.

If a man claiming to be "Doctor" W. W. Haid applies for work, communicate with Dr. R. G. Owen, 507 Stroh Building, Detroit, Michigan, who will be glad to furnish his record.

SACCHAROMYCOTIC TUMOR OF THE CLAVICLE*

JACK CLAYTON NORRIS

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Atlanta, Georgia*

For many years it has been known that yeasts were capable
of producing disease. According to Ricketts,[2] Reubold (1854)
found yeasts in the respiratory passages and Raum (1891) inocu-
lated yeasts into a rabbit producing death in the animal. Since
these writers made their investigations, numerous reports have
appeared and pathogenic yeasts are fairly well known. However,
very few articles dealing with yeasts as producers of inflammatory
change in bone have been published. According to Castellani,[1]
Vuillemin and Legrain (1900) reported yeasts as producing a sub-
maxillary bone tumor; Curtis (1896) found yeasts producing a
myxomatous tumor and Blanchard and Schwartz found yeast in
an abdominal tumor mass of a gelatinous type weighing a
kilogram.

CASE HISTORY

A colored male, eighteen years of age, was admitted to the hospital on Novem-
ber 8, 1930 with a complaint of "pain and swelling of right shoulder." In
August, 1930, he awoke one morning after a fairly comfortable night's sleep, and
noted that he had a slight dull and localized pain in the right shoulder region.
In a few days the pain was very severe. Following local applications, the dis-
comforture disappeared leaving a slight swelling. In September and again in
November the pain reappeared and he noticed the rapid growth of a tumor mass.
During the onset, and following the gradual sequence of above events, he never
to his knowledge had chills or fever. There was no history of trauma.

The physical examination revealed a fairly well developed male negro. The
pulse beat eighty per minute; the respiration was nineteen; and the blood
pressure was normal. In general his appearance was that of a nervous, very
weak, and very sick individual. The mucous membranes were very pale. The

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pupils reacted to light and accommodation. The teeth were in good condition. The tonsils were enlarged and cryptic. A few glands in the neck were enlarged. The chest was well developed and the excursion was almost normal except for a slight deficiency of the right apex. The bony landmarks were prominent. There was observed an enlargement of the distal extremity of the clavicle. This enlargement was bulb-like, beginning about the middle portion and gradually enlarging as it approached the distal end. In measurement it was approximately 8 by 6 cm. Palpation revealed this tumor mass to be very hard and bonelike, and at no point within the mass was there any softening. Just beneath the middle lower ridge there was a sinus which communicated with the bony structures and from which extruded blood-tinged material.

Within the contiguous tissues, especially posterior to the upper clavicular ridge, there was felt a softened area. Auscultation of the lungs revealed a few rales at the bases. There were also a few rales about the marginal bronchioles. The abdomen and extremities were normal. The laboratory examination revealed a blood count of 12,000 leukocytes per cubic millimeter. Seventy per cent of the white cells were polymorphonuclears. The hemoglobin was 50 per cent. On five occasions Wassermann and Kahn tests were made and in each instance were found to be negative. Smears for tubercle bacilli in the sputum and the sinus exudate were negative. Guinea pig inoculations from each failed to produce tuberculosis.

The roentgenological chest studies revealed a slight peribronchial infiltration. The studies of the clavicle tumor revealed a semi-cystic, semi-solid, productive and destructive change of the acromial part of the clavicle; the walls of the cortex were thinned out, and in two areas there appeared to be sinuses. The radiologist suggested that because of the structure and position and the absence of such changes in other bones, it was very likely that this disease was that of a benign giant cell tumor. This latter opinion was agreed on by two visiting orthopedic surgeons, and he was referred to the radiological department for roentgenologic treatment. The general opinion after a conference was that the disease was neoplastic of a benign type, but some favored the diagnosis of malignant sarcoma. In view of bacteriologic findings medical treatment was advised, based upon the fact that if it were a malignant tumor it had reached such proportions that it was inoperable and if it were benign there was no necessary haste in the matter of treatment.

I administered intravenously sterile solutions of gentian violet at weekly intervals beginning with 20 cc. of 0.5 per cent solution and increasing up to 35 cc. of 1.0 per cent solution. He received eight doses. After each dose the improvement was dramatic and without reactions. By mouth I administered saturated solution of potassium iodide up to sixty drops a day. He was alkalinized and fluids encouraged. On April 15, he was discharged perfectly well. The distal clavicular end was about the same size as that of the left clavicle. The report of the roentgenologist confirmed the curative observations.

BACTERIOLOGICAL STUDIES

A Gram stain and a Wright stain of the draining material each demonstrated the presence of polynuclears, giant cells, fibroblasts, and lymphocytes. Within the giant cells and within the polymorphonuclears were seen many Gram positive yeast-like bodies (fig. 1). There were no other bacteria present. Other smears revealed the same organism. Cultures yielding a yeast were made from material obtained from the draining sinus and also from pus removed by aspiration through penetrating a soft area just posterior to the acromial end of the bone. All cultures were made with the strictest aseptic technic. The media used
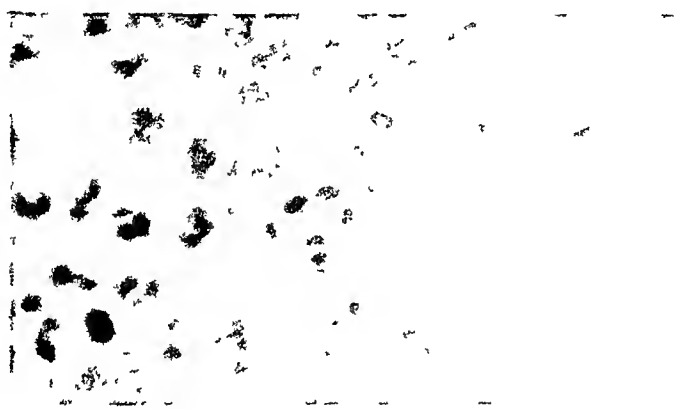
Fig. 1. Yeast Phagocytosed by Giant-Cell

were Sabouraud's, dextrose-beef-bouillon, plain and blood agar. On each of the mediums growths occurred after four days' incubation. The colonies, when very young, were very small and of a white pearly-gray color, turning darker as they grew older. The colony edges were smooth and from mother colonies other colonies were formed in a budding-like manner. The central portions of the growing colony were elevated and dome-shaped. As the colonies became older there was a very definite tendency to marked elevation and bubbles were noted. The colonies exhibited very little granularity but there was a viscid quality and the colonies tended to adhere. In some ways the growing colonies reminded one of a normal secretion from a mucous gland. The organism grew excellently aerobically but did not grow satisfac-

torily when all oxygen was excluded. It grew fairly well when
the culture was semi-anaerobic. Satisfactory culture results were
obtained on any of the ordinary acid enriched mediums. It did
not grow well on plain agar nor upon alkaline material. Slight
acid was formed in dextrose and sacchrose and very slight acid in
maltose. Gas was never present. Pellicle formation occurred on
maltose, dextrose, inosite, inulin and mannite. Litmus milk was
unchanged. Gelatin was not liquified. Endo's medium was
affected. Mannite agar was definitely resistant. On potato
there was a smooth, white, diffuse, elevated colonization. On
maltose there was a smooth, white, homogeneous growth. Sa-
bouraud's medium showed a whitish-gray homogeneous growth.
An interesting characteristic of the organism was noted when cul-
tures were made upon dextrose-beef-broth-agar; after several days
the progressively growing material tended to turn a brownish-
yellow color which gradually deepened as it aged. The organism
did not produce hemolysis.

CHARACTERISTICS AND LIFE CYCLE OF THE YEAST

In cultures grown on Sabouraud's medium for about a week the
organism was observed to be in various stages of division and to
be variable in size (fig. 2). They measured in size from 2 to about
30 microns. Unless they were in a state of division, the yeast cells
were perfectly rounded and practically all of the smaller ones had
within their central portions numerous granular-like bodies. As
the cell increased in diameter and approached its largest size,
there appeared within the central portion numerous very fine,
very small granules which had a Brownian-like movement. At
a later stage in the process of development, these dancing granules
shifted in an orderly manner toward the periphery of the ecto-
plasm. As this shift was noted, the movement of the particles
within the endoplasm tended to cease its activity and to become
quiescent; they were then larger and appeared to be more gran-
ular. With this change the large cell appeared to have a cellular
structure within a cell in which one portion of the inner ectoplasm
had a half-moon-shaped appearance. Next came the stage of
actual division in which there appeared at some portion of the

capsule an everted nippling.   This nippling gradually extended outward until finally it became spherical and detached itself from the parent cell.   Occasionally the daughter cell would extend its activity and an adjacent cell would be formed, sometimes three or four daughter cells being formed from one primary cell.

The quiescent cells in very old cultures showed bodies which might be construed as nuclei.   In anaerobic cultures which had been growing for a month or more the organism tended to reduce itself in size until it was very, very small, and in this state its characteristic features were represented by a small cell with a rather large, varying-sized nucleus.   In anaerobic cultures which had been growing for ten days or two weeks there were observed

FIG. 2. CULTURE OF *Saccharomyces pyogenes*

very unusual pleomorphic changes, in which these cells tended to change their size and shape, to elongate themselves and to become almost shadow-like and in comparison became shaped very much like sausages.   On several occasions I noticed the appearance at the extremity of these forms of a very fine mycelial hair-like process.   An attempt to cause mycelia to form by oxygen reduction in the culture was only partially successful.   It may be that these yeasts can form a very fine and unusual mycelium.   In anaerobic cultures such mycelia were formed and reproduced, yet in the animal I was never positive that it was recovered or that the mycelia played any part in the pathology.   I am certain that this yeast does not produce mycelia as is observed in

other pathogenic strains. The observations concerning the possible mycelioid forms in this yeast are important in that they may throw light on the biogenetics of these fungi.

The organism was destroyed after one hour's inactivation at 60°C. Two hours direct sunlight is necessary to destroy it. Agglutinins and precipitins are presumably not formed against it. One per cent phenol kills the organisms. A 1:100 mercurochrome solution, freshly prepared, will also destroy it: above this dilution the organism is unaffected. A half saturated solution of potassium iodide will inhibit the growth of the organism. Seven per cent iodine is an excellent germicide against the yeast. A fresh solution of gentian violet will destroy the organism in dilutions up to 1:31,000 approximately. This latter dye seemingly was the best germicide. Double sub-erythema unfiltered exposures with roentgen rays failed to influence the growth of the organism. The organism is Gram positive. Wright's stain and methylene blue are also excellent staining agents for it.

PATHOLOGICAL OBSERVATIONS

The most outstanding pathological changes in the patient were the very definite anemia, the leukocytic response, and the presence of a tumor mass. It is unfortunate that at first no section was made of the clavicular lesion. It seemed unwise to cut into the bone, fearful of results that might follow if it were a sarcoma. A section was obtained at the time the tumor mass had considerably disappeared, and the sinus had healed. The section showed a chronic inflammatory change in which there were seen a considerable number of fibroblasts of a reparative type, a few giant cells, proliferating bone cells, and a reparative fibrosis. There were one or more small areas where a previous necrosis had evidently existed. It was impossible to say that this tumor had not been a giant-cell type, yet I believe the lesion was purely infectious in nature (fig. 3).

In the animal, pathological observations were more comprehensive. Numerous animals were inoculated with the organism both intravenously and subcutaneously, and into the medullary cavities of bone. Guinea pigs developed hard nodular masses at the

site of inoculation in about three weeks.   Incisions into these
tumor masses did not show very much necrotic debris, and the
inflammatory change seemed to be largely one of a reparatory
nature.   The organisms were usually present and in every in-
stance were recovered by culture.   Sections showed the tumors to
be composed of fibrous tissue, leukocytes, lymphocytes, new form-
ing blood vessels, and an occasional giant cell (fig. 4).
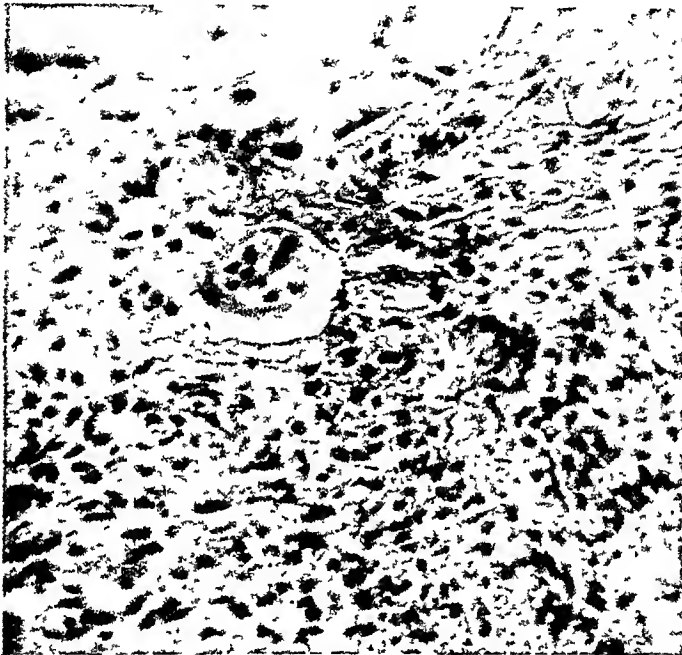
FIG. 3. SECTION FROM TUMOR.   NOTE GIANT-CELL

Rabbits inoculated were observed very closely for hemato-
poietic changes.   The rabbits had blood counts made prior to
inoculation.

Rabbit 212 was inoculated into the vein on November 24, 1930.   Previous to
inoculation his leukocyte count was 11,000.   Four days later it was 17,000.   On
December 9, it was 22,600.   On December 13, it was 24,800.   On the 15th of
December it was autopsied.   There was a general cloudy swelling and enlarge-
ment of the spleen, liver and kidneys.   The bladder was tremendously distended
and filled with urine.   Yeasts were present in the urine.

Rabbit 281 was inoculated December 13, 1930 with a culture of the organism
obtained from a previously inoculated guinea pig.   The leukocyte count reached

a peak of 20,800, sixteen days after inoculation. It, however, did not show a response until it was reinoculated into the medullary cavity of the left femur on December 22. It developed an abscess in the leg from which the yeasts were recovered. It also became paralyzed on the right side, and at autopsy meningitis was present; the organism was recovered from spinal fluid. This animal is
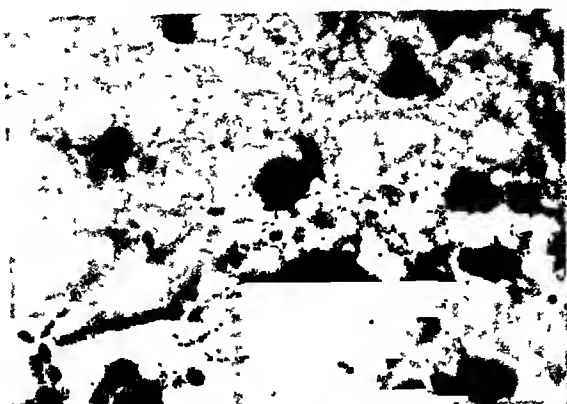
FIG. 4. YEAST IN THE TISSUES OF AN INFECTED GUINEA-PIG
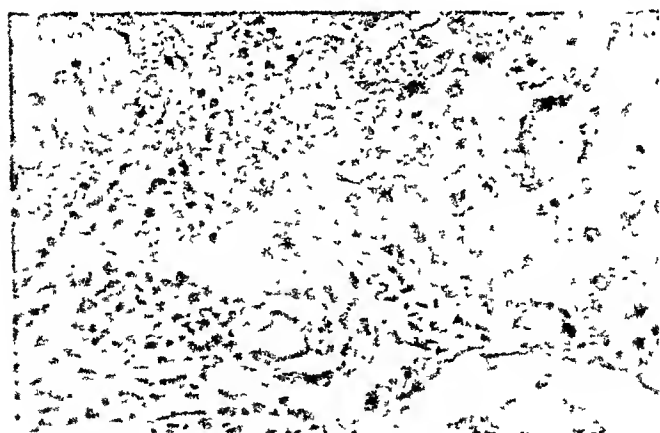
FIG. 5. SECTION FROM INFECTED RABBIT LIVER. NOTE GIANT-CELL

the most interesting of the group inoculated, and since the histological findings are very representative of those in the other animals observed, they are given in detail.

The liver shows a cloudy swelling of the fibers with a few scattered round cells in between them. There was also a fatty change taking place. The blood vessels were slightly thickened.

The *lungs* were moderately congested. The blood vessels were filled with a coagulable exudate. There were one or two areas which were semi-necrotic.

The *liver* (fig. 5) showed considerable cloudy swelling, a moderate fatty degeneration and thickening of the bile ducts and the arterioles. There was also present a round cell collection about the ducts and the vessels. The most interesting observations was the presence of giant cells scattered throughout the liver substance. They were present in considerable numbers. There were also present a few areas of latent focal necrosis with repair.

The cortex of the *spleen* was thick. The corpuscular areas were hyperplastic. There was a moderate cloudy swelling.

The *kidney* showed cloudy swelling in some areas of the tubules and degenerative changes in others. A few areas were semi-necrotic in type. The glomeruli were shrunken, and some were in a degenerative state; occasionally the arteriole was thrombotic. Casts were also present. Moderate fatty degeneration was also noticed.

The *brain* showed diffuse cloudy swelling with disintegration and atrophy of the ganglion cells. In some areas there were considerable numbers of lymphocytes and polymorphonuclears infiltrating into the brain substance proper. Occasionally a plasma cell was seen. There were also areas that were necrotic. Under the dura there were collected round cells.

The yeasts were positively identified by Gram staining as existing in the tissues of this animal.

Rabbit 216 was inoculated into the medullary cavity and into the blood stream. The left leg was used as control. This rabbit ran a persistent leukocytosis, averaging 20,000. It developed a very definite periostitis which existed from December 9 until March. The animal's leg was definitely enlarged, and the radiological studies showed new bone formation within the cavity. At the time autopsy was performed this tumor mass was receding and nothing can be claimed for more than a very definite inflammatory reaction.

In summarizing the histological features in the animal, the conclusion is reached that the infection is characterized by giant cell proliferation, leukocytic response, cloudy swelling, fatty degeneration, fibrosis and necrosis. Although no tissue is immune, there seems to be a selective affinity for brain substance. A further conclusion is that the organism does not produce acute disease changes in the strict sense of the word, and more often produces chronic and subacute lesions. Even the animals which developed meningitis many days after inoculation did not present symptoms of an acute meningeal involvement. The animals characteristically were paralyzed on one side. As the culture grew older its virulence became lessened.

ARGUMENT FOR ETIOLOGIC INCRIMINATION

The following facts are offered as sufficient to support the claim that this organism was the etiological agent in the disease:

1. The presence of yeasts in the exudate coming from the tumor.

2. The absence of other germs of any type or description, as proved by repeated cultures, anaerobically and aerobically.

3. The presence of the yeasts in the giant-cells and within the polymorphonuclear leukocytes.

4. Recovery of the yeast on fifteen different occasions by culture.

5. The demonstrations of its pathogenicity for animals with the production of a periostitis and other phases of inflammation; the recovery of the organism from the lesions.

6. The serologic observation that the serum of the patient when placed with another individual's leukocytes caused a remarkable ingestion of these yeasts by the leukocytes, although control serum would not give rise to such phagocytic action.

7. The recovery of the patient when treated with chemicals which destroyed the yeasts in vitro.

SUMMARY AND CLASSIFICATION OF THE YEAST

This report is made because of the rarity of such lesions and to suggest particular study of bone tumors of unknown diagnosis and particularly of the benign giant-cell and cystic types. It is within the realm of possibility, and many authorities believe this to be true, that such lesions are due to infectious agents which may respond to treatment more easily given and not so disturbing as surgery. Another reason for the report is to add further evidence of the importance of yeasts as disease producers. The treatment in this instance while radical is seen to be rational and productive of excellent results.

Castellani does not list this yeast. It differs from the pathogens described by Stoddard and Cutter, Blanchard and Schwartz, Vuillemin and Legrain, and by Curtis.

Thus I feel it proper that the new name *Saccharomyces pyogenes* be given it. It should be classified under the Family Saccharo-

mycetaceae and Genus Saccharomyces which contains yeasts not forming typical mycelia.

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STUDIES IN CEREBROSPINAL FLUID. I*

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The cerebrospinal fluid being a product of the choroid plexus, arachnoid, et cetera, structures intimately related to the central nervous system, both morphologically and physiologically, may naturally be expected to reflect in its properties pathological involvements of this system. An examination of the cerebral fluid has thus become a routine procedure in the clinical diagnosis of many conditions, and numerous procedures have been developed, aiming to detect and differentiate the various changes that take place in certain diseases of the nervous system. With the exception of the Wassermann test, which is based on a reaction specific for syphilis most of the other tests are chemical or physical, depending on the difference in concentration of some constituents of the fluid in normal and pathological conditions. An examination of the actual differences in chemical composition between cerebrospinal fluid from normal and pathological individuals, however, shows a distinct and definite change of any magnitude to occur in regard to only one colloidal compound, namely, protein. Nor is this very surprising when the nature of the fluid is considered. It contains normally very little protein, and contains salts in concentrations related to the composition of the blood, most probably according to the simple physicochemical laws of distribution across a membrane. It is to be expected, therefore, that any condition involving the meninges or other sources of spinal fluid should affect its protein content, regardless of whether its formation is considered to be one of simple filtration, secretion, or excretion. The composition of other constitu-

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ents will also naturally change, but not in so marked a manner as that of the proteins, as analysis has demonstrated. Examination of the methods that have been suggested for examination of pathological fluids, will show that they depend on the increase in the protein content of the fluid. Thus the foam test described by Levinson is dependent on a high content of protein. The various precipitation tests, the colloidal gold reaction of Lange, the mastic test of Emmanüel, are all probably dependent on a high protein content of the fluid for their value as diagnostic agents. The same is true of Pandy's, Ross-Jones' and Noguchi's tests, all of which are protein tests.

Of all these methods, the Lange test stands out as the most valuable diagnostic procedure, but it suffers the disadvantages of being cumbersome, of requiring a rather elaborate technique and scrupulous care in its use and of being easily invalidated. This paper describes a simple test which depends on the protein content of the spinal fluid and which being of extreme simplicity in regard to ease and rapidity of manipulation can act as a useful means for the laboratory diagnosis of abnormalities in the spinal fluid.

PROCEDURE

Ten Wassermann tubes are set up in a rack. Into tube 1 there is pipetted 0.3 cc. of spinal fluid, into tube 2 there is pipetted 0.23 cc. of spinal fluid. To tube 1 there is added 0.7 cc. of physiological saline, and to tube 2 is added 0.77 cc. of saline, bringing the volume in each tube up to 1.0 cc. Into each of the other eight tubes 0.5 cc. of saline is added. The fluid in tube 1 is well mixed, by gently shaking or by pipetting, and 0.5 cc. is transferred to tube 3, mixing well and transferring 0.5 cc. to tube 5, and so on in alternate tubes until the ninth tube is reached, from this tube 0.5 cc. is discarded. Then beginning with tube 2, the fluid is well mixed and transferred similarly in series to tubes 4, 6, 8, 10, discarding the 0.5 cc. from tube 10.

To each tube is then added 1.0 cc. of starch-iodine solution. The tubes are carefully agitated until the color is uniform in each tube, and the color of each tube is read immediately. The final readings are taken one-half hour later.

The starch-iodine solution is made as follows:

One part aqueous iodine (0.1 gram in 1000 cc. distilled water).
One part starch solution (0.75 gram in 100 cc. of saline).
One part physiological saline (8.5 grams NaCl in 1000 cc. of distilled water).

One-tenth gram of fine iodine crystals will dissolve in the water in from three to four weeks. The water must be absolutely free from organic matter so that the solution of iodine will not be weakened by any reaction or standing. Soluble starch is used for the starch solution and is heated only until a clear solution is obtained. The solution need not be boiled and should not be used when it has become cloudy on standing. The glassware for the test should be chemically clean and dried by sterilizing in the gas oven, thus keeping it free from foreign matter, such as lint, dust and the like.

The readings are made in symbols as follows: decolorized tube, O; light blue color, L; standard blue of starch-iodine solution, B. Enclosing the letter in parentheses indicates a lesser value, thus, (L) indicates a very pale blue color and (B) a slightly affected affected standard blue color.

In normal fluid two tubes are slightly affected, and one tube is sometimes decolorized.

In tabes, from four to five tubes are affected and two of these are decolorized.

In paresis, from five to six tubes are affected and three of these are decolorized.

In meningitis, from seven to nine tubes may be effected, according to the severity of the case and from five to six of these are decolorized.

It is interesting to note that if there is a clinically appreciable improvement in treated cases of meningitis, paresis, or tabes, the same number of tubes is affected, so that as long as the condition prevails the same number of tubes is decolorized, but to a lesser degree, the colors in the tubes being fainter than in the untreated cases. This characteristic has been especially noticed in cases of meningitis.

It has been observed that when a pellicle, or coagulum has formed in a spinal fluid the reaction does not attack the usual number of tubes, but that a curve is obtained in which two tubes less than the usual or typical number are decolorized. That this condition also affects the colloidal gold readings is shown in the table which gives comparisons between the typical Lange colloidal gold and the Gruskin tests. In the formation of the pellicle, it would seem that a considerable amount of the proteins are held together and thus kept from entering into the reactions.

In nearly 200 spinal fluid tests, the results have checked with the Lange colloidal gold test in all but one, which was a case of treated syphilis, in which the Gruskin test did not coincide with the Lange. The tabulation gives a few of the results in order to show the various types of curves obtained with the test.

COMPARISON BETWEEN LANGE AND GRUSKIN TESTS
(Typical tests)

DIAGNOSIS	LANGE	GRUSKIN
Normal	01000000000	(B)BBBBBBBB
	01100000000	L(B)(B)BBBBBBB
Tabes	12222211110	OOLL(B)BBBBB
	11124432100	OOL(B)BBBBBB
Paresis	5555544100	OOO(L)(B)BBBBB
	5555555321	OOOL(B)BBBBB
Meningitis	11111343210	OOOOOOL(B)(B)B
	12222333210	OOOOL(B)BBBB

THEORETICAL CONSIDERATIONS

The theoretical basis of the test is dependent on the behavior of colloial proteins in solution. There are several theories concerning protein behavior each of which have logical arguments in their support, but none of which as yet has been unanimously accepted or established in stoichiometrical form.

Thomas Graham was a pioneer in the study of the visibly amorphous state of matter which he suggested calling colloidal, as opposed to matter with a definite crystalloidal structure. Also he was the first to suggest the possibility of the same substance occurring in both the colloidal and crystalloidal states. He[3] wrote "The inquiry suggests itself whether the colloidal molecule may not be constituted by the grouping together of a number of smaller crystalloidal molecules, and whether the basis of colloidality may not really be due to this composite character of the protein, or other colloidal molecule." Modern research has fixed the dispersion into particles between 100 and 5 $\mu\mu$ as the

criterion of the colloidal condition, and the particles may be crystalline or consist of random clusters. But these statements of Graham still hold true.[6] If this suggestion were so, the logical consequence would be that colloids should be governed by laws analogous to those of the crystalloidal systems. This has been lost sight of for a considerable time and it is only recently that the analogy has been again under consideration.

Grolman, working with phenolsulphonephthalein, claimed that the substance was taken up by serums and aqueous solutions of varying amounts of proteins, according to the Freundlich isotherm

$$\frac{x}{M} = \alpha\, C\, \frac{1}{n}$$

where x is the weight of substance adsorbed by M, the weight of the adsorbent, C is the volume concentration at equilibrium, and α and $\frac{1}{n}$ are constants varying only over a wide range. Loeb[5] attempted to refute this theory on the charge that the observers did not consider the pH of the protein solutions under consideration, thus obtaining inconsistent results with the variation in pH. Loeb adhered to the chemical view that proteins form true ionizable salts, for example, gelatine chloride and sodium gelatinate. On the other hand, Loeb's theories have been contested on the ground that he did not demonstrate protein, namely, the gelatine used by him, as a definite chemical entity, and that he was not sufficiently specific as to quality and composition. Robertson[6] has drawn the following comparison: our knowledge of the colloidal character of proteins, especially concerning their indiffusibility or slight diffusibility, has created the impression that in solution they will furnish surfaces at which adsorption may occur. It is inferred and granted that the ultimate particles of protein in solution are very large in comparison with molecules of crystalloidal substance; from this the conclusion is drawn that such large particles would present a surface to the surrounding medium at which adsorption might occur. But, he adds, if the chemical constitution of the proteins is considered, it is quite

evident that the ultimate chemical unit of protein, the molecule itself must be very large. As an example, the proportion of iron in hemoglobin indicates a molecular weight of 16,000, that of tryosine, glutamic acid, and cystine in casein indicates a molecular weight of from 4000 to 4400; phenylalanine in gelatine indicates a weight of 11,800. Thus it is evident that the colloidal properties of proteins must naturally follow from the magnitude of their molecules. Therefore the ultimate surface which separates the protein from the solution must be the molecular surface in the protein as well as in other substances, for it has been demonstrated that in nearly all instances single or double molecules serve to account for the colloidal behavior of protein solutions. Therefore since adsorption at the surface of the molecule of sodium chloride, water or sugar is not spoken of, why should this be attributed to the protein molecule alone. If adsorption did exist at the molecular surface would it be distinguishable from chemical combination? Then should the same phenomenon be called chemical combination when it involves a small molecule and adsorption when it involves a large one? On the other hand, in a recent study Field[2] claims that the significance of dilution as a factor in determining the iodine binding capacity of starches suggests that starch-iodide is an adsorption compound. A lack of specificity towards the combination with iodine shown by the various starches also seems to point towards adsorption.

At any rate it is now admitted by all observers that the proteins accomplish the neutralization of acids and bases in stoichiometrical, that is molecular or equivalent molecular, proportions.[6] Many refuse to admit a purely chemical mechanism and invoke a physical mechanism, that is, molecular attraction between the colloidal particles of protein and the molecules of acid or base which are neutralized.

In the reaction of the blue starch-iodide mixture with the proteins of the spinal fluid, the sensitiveness of the test is dependent on the affect of the proteins on the iodine, the starch serving the purpose of forming the blue complex but evidently not reacting with the proteins themselves. This is demonstrated in positive cases of reaction where the color is discharged, for

an addition of starch does not renew the color, whereas introduction of a sufficient amount of iodine does again produce a blue color. Inorganic constituents had a negligible effect on the reaction so that it is a specific test for proteins. It has been pointed out by Chesley[1] that starches from different makers give different results in their digestibility with enzymes, as tested with the iodine reaction. It is therefore advisable to avoid variation in readings of this test that the starch of one company be used constantly. In this manner any inaccuracies from the use of starches of different values will be eliminated.

Regarding the sensitiveness of the test, it is well to note that procedures involving the starch-iodide reaction offer possibility of great delicacy, inasmuch as by this reaction it is possible to detect as little as 0.0000001 grams of iodine per cubic centimeter of solution.[4]

SUMMARY

A simple colorimetric diagnostic test for spinal fluid has been described. Only a small amount of fluid is required. The procedure is devoid of the usual difficulties of the other tests, and since it is based on a sound theoretical mechanism, it is hoped to have a diagnostic value in the routine examination of the cerebral fluid superior to many of the more complicated tests in use at present.

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THE THERAPEUTIC USE OF BACTERIOPHAGE AND ITS PRACTICAL DIFFICULTIES*

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The mechanism of bacterial lysis is still so little understood that no one can state dogmatically what bacteriophage is. For the purpose of this brief discussion bacteriophage may be defined as that principle which brings about transmissible lysis of bacteria; bacteriophage also increases or multiples during that process. It is not necessary to go into the question of whether bacteriophage is a virus, an enzyme, or a product of autolysis. It has properties suggestive of each of these. Most of the facts known concerning bacteriophage would apply to a virus, and this explanation is more easily comprehended than the explanation of the phenomenon upon purely physico-chemical grounds.

Whatever shall finally be found to be the true nature of bacteriophage, it certainly has not been decided at the present time, and it is not within the province of this review to try to settle it. It is my purpose to point out the principles underlying bacteriophage therapy, and some of the causes of failure of this form of treatment.

The lysis of a culture in vitro by specific bacteriophage is so spectacular, that we were justified in our hopes and expectations that the same thing would take place in the animal body harboring pathogenic organisms.

Experimental studies have indicated that the course of an experimentally induced infection can be favorably influenced by a bacteriophage capable of lysing the causative organism.

The results of the clinical studies have possibly been a little more optimistically interpreted than have the experimental data, as it is quite impossible to subject clinical studies to the same rigorous methods as are applied to experimental work.

The infections in which bacteriophage is of value are, as a rule, those in which the infectious process is largely on or near the surface. For example, the most brilliant results reported from this method of treatment are in cholera. The results reported by d'Herelle[1] in this disease are more spectacular than those reported by others in any condition.

There is practically a unanimity of opinion of those reporting upon the use of bacteriophage in the treatment of staphylococcic infections, that the method is of value. This was brought out by Larkum[2] in an analysis of some 700 reported cases. It is possibly a mistake to try to evaluate the results of bacteriophage in all types of staphylococcic infections grouped together, because of the great variety of lesions present. The accessibility of the staphylococci in an open wound infection, for example, is quite different from that in an abscess, a paranasal sinusitis or an osteomyelitis.

This is probably the principal cause for failure in treatment of staphylococcic infections. The accessible staphylococci can be readily lysed by bacteriophage, and with less tendency to secondary growth than is the case with most organisms. This is apparent in the treatment of staphylococcic wound infections, which are usually benefited by bacteriophage therapy.

The following case brings out a point of considerable importance.

The patient had had an amputation below the knee. The stump became infected with *Staphylococcus aureus*, and after other measures had failed the wound was treated with staphylococcic bacteriophage. Improvement was immediate but when the treatment was changed to a mixed commercial preparation of colon-staphylococcus phage, a secondary infection with *Pseudomonas aeruginosa* (*B. pyocyaneus*) occurred. On subsequent examination it was found that the mixed phage was contaminated with live organisms. These broth filtrates are perfectly satisfactory for the growth of heterologous organisms, and great care should be exercised to prevent their contamination, not only during preparation but also at the time of applying.

In typhoid fever and dysentery, bacteriophage therapy has apparently been unsatisfactory. I have had little experience with it in typhoid fever, and none in dysentery.

In the colon bacillus infections the results have been very variable. Some of the reports of bacteriophage treatment of pyelitis have been favorable, but many of them have indicated little or no effect. I have seen one case of pyelitis in a young girl in which the result was very gratifying, but for the most part the results in this condition have so far been only fair. The best results in infections due to the colon bacillus have been in those of abdominal wounds.

Pelouze and Schofield[3] reported the development of a bacteriophage in lysed cultures of gonococci, but this substance was without therapeutic effect in gonococcic infections. Bacteriophages have been encountered that were effective against most of the common infectious organisms in the test tube, but most of these have been ineffective in the body.

Several factors are responsible for the variability in the therapeutic effect of bacteriophage. First, as mentioned above, the accessibility of the pathological focus to the bacteriophage filtrate, is of primary importance.

Second, if a bacteriophage does not completely destroy a culture, the organisms that survive give rise to a resistant strain of organisms. These may still be pathogenic for the host but unaffected by the bacteriophage. There is available for staphylococcic infections a bacteriophage which is lytic for practically all strains of staphylococci, and is so potent as to destroy many strains without the development of resistant forms. Colon, typhoid, and dysentery bacilli, on the other hand, develop resistant strains much more readily. Only young cultures of most strains of these organisms are susceptible to complete lysis, and the older organisms become the progenitors of resistant strains.

Third, bacteriophage is not very effective against blood borne infections, because of the adsorption of the phage by the colloids of the serum. In some manner not well understood the bacteriophage sometimes becomes free from the colloid and localizes at the site of infection. It would be difficult to explain on any other

basis the fact that bacteriophage is to some degree effective, in experimental animals at least, when injected at a site distant from the infective focus.

Fourth, it has been found[4] in certain experimental infections, as in peritonitis for instance, that to get the effect of bacteriophage, it must be injected before or within a half hour after the bacterial inoculation. This condition cannot be fulfilled in clinical practice, and in such infections the use of bacteriophage will have to be limited to prophylaxis for the present. Some experimental data encourage us to hope that the prophylactic use of bacteriophage may prove of some value in these conditions.

Finally, the protein present in a bacteriophage filtrate has an important bearing on the dosage and method of choice for administration. The protein is derived, from the extractives in the culture mediums, and from the bacterial protein of the lysed organisms. When bacteriophage is to be administered by mouth, as in cholera, typhoid fever, dysentery, et cetera, the protein present in a dose of 2 to 10 cc. is of no significance, but if it is to be injected intravenously the amount of peptone and bacterial protein in the filtrate becomes a matter of major importance. To a lesser extent this is also true of subcutaneous or intramuscular administration. I have seen a very severe and extensive local reaction follow a dose of what evidently was a too highly concentrated bacterial protein.

Bacteriophage may be administered by mouth, by irrigation, by wet dressing or by inoculation. The administration by mouth is the method of choice in cholera, typhoid, dysentery, et cetera. I have administered it by mouth in pyelitis also, in the hope that some of the phage might be excreted by the kidney. When bacteriophage is given by mouth it does not cause the development of an antibacteriophage which is a decided advantage. From 2 to 5 cc. of an active phage filtrate may be given daily over a period of from seven to ten days.

Wet dressings of bacteriophage sometimes have a spectacular effect on wound infections. I have seen this both in staphylococcic and in colon bacillus infections. Bacteriophage usually from 1 to 4 cc., is poured into the wound, and a wet dressing of physiological salt solution is applied.

Probably the commonest method of administration of bacteriophage is by subcutaneous inoculation. This may be given intramuscularly or intravenously. The latter method is seldom used because of the adsorption of bacteriophage by the colloids of the blood, as mentioned before. The filtrate is usually injected directly into the lesion, but at least in certain experimental infections, it may act on a distant focus. This was shown by Walker[4] in peritonitis in mice. Bacteriophage may be injected directly into and around a boil or carbuncle. The dose varies from 0.1 to 1.0 cc. according to circumstances. In pyelitis the method of choice is irrigation of the bladder, and possibly the pelvis of the kidney.

It would be desirable to have a more definite method of standardization of bacteriophage filtrates. Various authors have specified their dosage simply according to the quantity of filtrate used, or by the number of phage corpuscles in a dose, or by the number of lysed organisms in a dose.

For the present it is probably best to designate the number of corpuscles in each cubic centimeter of filtrate, and adopt some arbitrary number, as a billion in each cubic centimeter, as a unit.

When more is known of the chemistry and antigenic properties of these filtrates of lysed organisms, it may be found that a large part of their therapeutic or prophylactic value is due to the bacterial protein, and that standardization on the basis of the number of lysed organisms is more logical than the present method.

Bacteriophage filtrates have failed to justify our hopes that they would prove a panacea for infectious diseases, but there is reason to believe that they have a definite place as valuable therapeutic agents.

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THE NATURE OF HODGKIN'S DISEASE

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The origin of Hodgkin's disease has always been confusing. We shall attempt to show that the disease is probably neoplastic and that it belongs to the group of lymphoblastoma.

A variety of names has been applied to this condition, such as multiple lymphadenoma, lymphogranuloma, lymphoblastoma, lymphocytoma, malignant granuloma, infectious granuloma, lymphosarcoma, and pseudoleukemia. Mallory,[9] and MacCarty[8] each suggested that all these terms be grouped under the heading of lymphoblastoma, which would indicate any tumor composed of cells of the lymphocytic series. This group would also include all forms of lymphatic leukemia. Warthin[15] selected the name lymphocytoma to designate all hyperplastic tumors and those that resemble neoplasms of the lymph nodes, depending on an overgrowth of cells of the type of small or large lymphocytes.

Ewing[3] stated that a consideration of lymphomas should include the various processes by which they are produced, for some of them are inflammatory, some neoplastic, and others intermediate in position. Lack of knowledge of the etiologic factors and of the intricate relation of many forms of lymphoid hyperplasia, and the occasional transformation of one form to another, makes a classification on an anatomic basis justifiable. Even in this difficulties are encountered because the relation of the lymphocytes and the reticulum cells has not been definitely determined, and the distinction between sinus cells, endothelial cells, and reticular cells is not definite.

Thomas Hodgkin,[6] in 1832, described a clinical syndrome in which there was swelling of the lymph nodes, which syndrome was designated by Wilks as Hodgkin's disease. In the following years

many terms, as mentioned previously, were applied to this condition. Until Sternberg[12] described it as a peculiar form of tuberculosis, most investigators looked on the disease as a neoplastic condition. Reed[10] then gave an accurate description of the lesion, which has changed the status of the condition from that of a clinical syndrome to that of a pathologic entity. However, since that time, numerous articles have been written dealing with various phases of the malady, but not a single conclusion has been reached regarding its true nature. There is still much disagreement as to whether the lesion is a neoplasm or a granuloma resulting from an inflammatory process.

Tuberculosis as a probable excitant has been widely commented on. Sternberg at first emphatically maintained that Hodgkin's disease was of the same etiology as tuberculosis. Later he modified his position and admitted the existence of nontuberculous forms. Fraenkel and Much[5] demonstrated Gram-positive bacilli and acid-fast granular bacilli in twelve of thirteen cases of Hodgkin's disease, and suggested that these were certain forms of organisms of tuberculosis. Ziegler[16] also pointed out that the condition in 20 per cent of the cases was associated with tuberculosis and that in 10 per cent the reactions to inoculation were positive.

Sticker[13] reported that the bovine type of the organism of tuberculosis was found in the lymph nodes after repeated passage through guinea pigs. L'Esperance[7] inoculated chickens with emulsions from the lymph nodes and typical or atypical tuberculosis developed in all: inoculation of other chickens resulted in even more extensive manifestations of the disease. She concluded that the avian type of bacilli might be important in the production of Hodgkin's disease.

Sweany[14], stated that the disease pursues the typical course of an infectious condition, and that the organism of tuberculosis might degenerate and pass over into the diphtheroid type that was practically a distinct species: he stated that he had been able to produce such forms. He also stated as his belief that tuberculosis of the lymph nodes often developed into Hodgkin's disease, and that the whole picture was a heterogeneous degeneration of an

atypical strain of organisms of tuberculosis in a particular type of host that had failed to overcome the parasite.

Stahr[11] expressed himself in favor of the view that the lesion was of the nature of a tumor with organisms of tuberculosis as an excitant. He stated that for the production of a tumor the stimulus must not be strong enough to break down the defensive forces of the body. The inflammation need not be specific, but it was essential that the irritation be mild and of long duration. He stated as his belief that when the defensive efforts of the body were not aroused, enormous hyperplasia might be present.

Syphilis has never been seriously considered as a cause of Hodgkin's disease, but it has been mentioned by Fabian[1] and other older observers. However, a positive Wassermann reaction has never been obtained except in cases in which syphilis was present independently.

It can readily be seen how difficult it is to apply any definite designation or classification until the etiology of this group of conditions becomes known. Staining of sections for the organism of tuberculosis has failed to reveal it, but in one case tuberculosis was associated. Tuberculosis with hyperplasia and without caseation, sometimes simulates Hodgkin's disease. The fact that the node filters lymph makes it most commonly involved secondarily, and it is very difficult to determine whether one is dealing with a neoplasm or a granuloma. In the intermediate types it is even more difficult to determine whether the process is strictly inflammatory or neoplastic. Productive inflammation usually involves all the elements of the node, whereas a neoplastic process usually involves a single cell type; yet is should be remembered that in the same person hyperplasia may remain simple in one situation, but take on neoplastic properties in another situation and that infection may play a part similar to the one it plays in carcinoma. Theoretically there may be a point at which the transition occurs between inflammatory hyperplasia and neoplastic hyperplasia. Thus it may be seen that the numerous types of lymphoma described may be different stages or manifestations of the same disease.

The material used for this study consisted of 200 cases of Hodg-

kin's disease in which one or more cervical lymph nodes were removed as a part of treatment or for diagnosis. All of these cases were observed and the patients were treated at The Mayo Clinic between 1910 and 1920 and they have been traced and their subsequent condition noted. In this series the youngest patient was a boy aged four years, and the oldest patient, a man aged sixty-five years. The duration of the disease was from six months to seventeen years, with an average of two and sixty-five hundredths years. The disease occurred much more frequently in males. The response to roentgen therapy was practically the same as that of lymphosarcoma.[2]

The nodes were usually enlarged, and sometimes measured 4 cm. in diameter. They might be round, oval, or oblong, and might have a distinct capsule, which was frequently thickened. The nodes, in an involved group of nodes, were discrete. On gross examination the fresh specimen was salmon-pink, homogeneous, and had a consistence resembling rubber. Its appearance was sometimes altered by the duration of the condition, degenerative changes, and previous treatment. In the nodes that grew very rapidly, or that were most malignant there might be some central necrosis. Those that grew more slowly contained more fibrous and hyaline tissue, thus altering their color and consistence. Previous roentgen therapy might also produce similar changes. The capsule was rarely, if ever, invaded by the neoplastic process. On microscopic examination, the nodes in different cases varied in appearance, depending on the degree of malignancy present.[1]

The disease began in the so-called germinal centers, the cells of which are derived from the wandering histiocyte which in turn is derived from the fixed histiocyte, or what might be called the stem or parent cell. Thus, Hodgkin's disease and lymphosarcoma are derived from the same stem cell, but differ in the degree to which this cell becomes differentiated. The second parent cell of Hodgkin's disease is the free or wandering histiocyte, whereas the second parent cell of lymphosarcoma is the lymphoblast. It would be possible for the two conditions to be associated.

In the first stage there was extensive catarrhal inflammation of

the sinuses in the form of hyperplasia of the lymphoid cells, with active proliferation of the germinal centers. Vascularity was also increased. The sinuses were dilated and contained small and large lymphocytes, polymorphonuclear leukocytes and eosinophils. In the reticuloendothelial cells, mitotic figures might be found. The reticulum was prominent, and large lymphocytes,
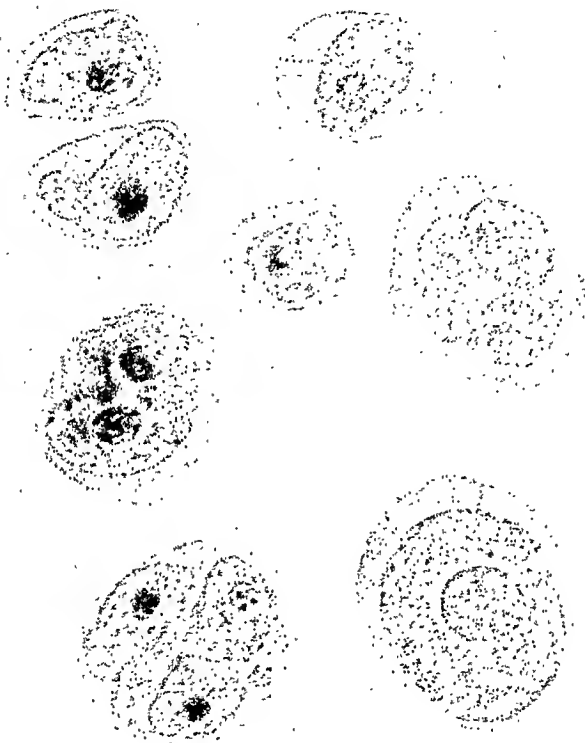
Fig. 1. Single and Multinucleated Cells Characteristic of Hodgkin's Disease. Drawings from Photograph. (×1100)

epithelioid cells, plasma cells, mast cells, and eosinophils were often distributed profusely.

In the second stage the appearance was more striking. The thickening of the reticulum became more noticeable. A coherent tissue appeared which was formed of large cells, with elongated, vesicular, and rather palely staining nuclei. The cells lay in no particular order, and with other lymphoid cells soon spread to

place the normal tissue, and to obliterate the distinction between lymph cords and sinuses. Here and there the remains of lymph follicles might be seen. The capsules were thickened, but distinct narrow bands of connective tissue divided the node into irregular lobules, and fibroblastic proliferation became more prominent.

The multinucleated giant cells formed a fairly constant picture. They were probably formed by the unicellular giant cells which in turn are derived from the reticular endothelium. The uninuclear giant cell was very irregular in form, and had a clear protoplasm, containing a large nucleus, which might be multilobular and might contain one or more nucleoli. Surrounding the nucleus was a fine, deeply staining portion of chromatin, and in the center, a well defined network, with one or several deeply staining and sharply defined nucleoli. The nuclei numbered from 4 to 10, and were heaped together in the center of the cell. Occasionally there was a second form in which the nuclei were shaped in the form of a horseshoe around the periphery of the cell. This type resembled closely the Langhans giant cell of tuberculosis. The nuclei resembled those found in foreign-body giant cells.

The cellular condition found in the earlier stage gave way to progressive scarring throughout the node. Finally, a dense mass of fibrous tissue remained, in which there were nests of cells, such as have been described. Cellular degeneration took place, and not infrequently regions of necrosis were found. These regions were infiltrated with fibrin, and were surrounded by leukocytes. Cells might undergo fatty degeneration, and hyaline degeneration occasionally took place.

Eosinophils might be uninuclear or polynuclear and were rarely absent. Infiltration by them has attracted much attention; any condition which results in the destruction of lymphocytes will bring about this invasion. The exact relation of these cells to the process has not been determined.

The characteristic cell was large, with clear cytoplasm containing fine reticulum, and the so-called nuclear membrane was distinct. The nucleolus was oval or round, fairly large, centrally situated and stained deeply. In the more rapidly growing cells

many bizarre nucleoli were seen, which stained deeply. Tumor giant cells that usually contained from 2 to 5 nucleoli also accompanied the more malignant types. Germinal centers were necessarily absent, but in earlier cases some still might be present.

Eosinophils, plasma cells, and occasionally foreign-body giant cells occurred, and produced a granulomatous appearance. However, this reaction, accompanied usually by fibrosis and hyalinzation, was a defensive mechanism that varied with the subject, and the degree of malignancy.

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NOTE ON THE SPECTROSCOPIC TEST FOR SULPHEMOGLOBIN

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Within the last few years methods adapted to the use of the hand spectroscope have been developed for demonstrating methemoglobin and sulphemoglobin in blood.[1,2,3] By these methods the presence of the pigments can easily be shown if their concentrations are 1.5 volumes per cent or higher, that is, definite positive results are obtained if 10 per cent or more of the blood pigment exists in an abnormal form. Concentrations below 0.7 volumes per cent are rather difficult to demonstrate by simple methods, particularly if oxalated or citrated blood samples are examined, unless the technician has had considerable experience in such determinations.

Recently a case of cyanosis which was obviously due to poisoning from freshly dyed shoes occured in a nurse in this hospital. We were not able to find any abnormal pigment in the blood, although we felt sure that it was present. This experience led us to attempt to standardize technique to avoid similar errors. It was decided to study the determination of sulphemoglobin because the general importance of this compound seems to be greater than that of methemoglobin, and because the former pigment can be more readily prepared than the latter one. The close resemblance of the two pigments to each other made it seem probable that a method for demonstrating one of them would in general be applicable to the other.

For these experiments large amounts of oxalated blood were pooled and sulphemoglobin was prepared from part of the material by running a current of hydrogen sulfide gas through it for half an hour or more. The excess of hydrogen sulfide was then removed as completely as possible by aeration. Such prepara-

tions gave the characteristic spectrum of sulphemoglobin. They contained, at most, minimum concentrations of free soluble sulphydryl compounds, because negative sodium nitroprusside reactions were obtained when such tests were carried out on the filtrate after the protein was removed with trichloracetic acid. The blood containing sulphemoglobin was mixed with untreated blood in various proportions. Saponin was then added to the samples, and they were allowed to stand for five minutes or more until hemolysis was complete. Next they were centifuged at
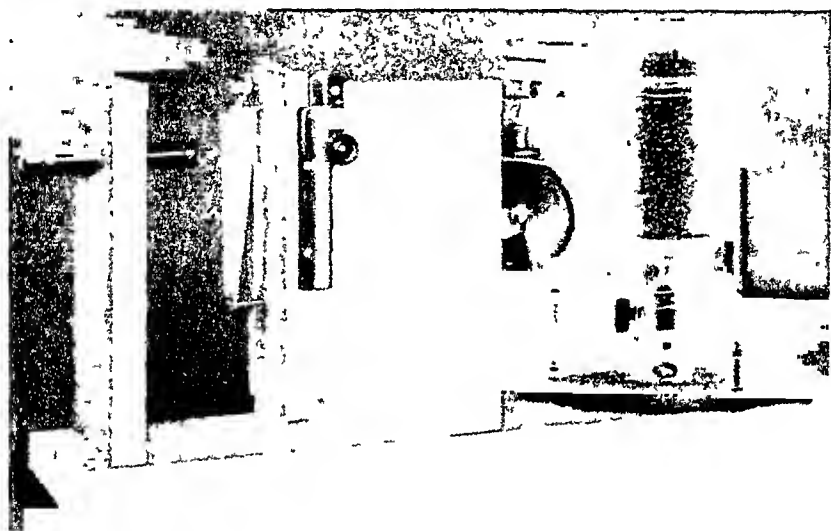
Fig. 1

high speed to remove the excess of saponin and the cell rests. They were then diluted with an equal volume of distilled water and examined with a hand spectroscope in tubes of one-half to three-quarters of an inch in diameter. Results were somewhat more satisfactory when a very bright light equipped with a round-bottomed flask as a condenser was used, but such a system was not necessary except for the detection of very small traces of pigment. The only light which came through the solution was in the red and orange portion of the spectrum, and the characteristic band of sulphemoglobin could be easily distinguished when only 2 per cent

of the hemoglobin was in the altered form, that is, when the concentration of sulphemoglobin (expressed in terms of the unaltered pigment) was 0.3 grams per 100 cc. of blood. By careful comparison with normal blood it was possible to detect amounts approximately half as great as these, but the reading of such concentrations was difficult. When the sulphemoglobin formed was more than 20 per cent of the total pigment its absorption band was so wide that it fused with the general absorption produced by the unaltered hemoglobin. Such solutions, which are easily recognized by their purple-brown color, are either diluted with water, about five parts of water to each part of the diluted, hemolized blood, or are placed in tubes of very small diameter for examination. If the hemolized blood is poured into a conical centrifuge tube it is easily possible to vary the thickness of the layer of solution through which light passes to the spectroscope. This technique not only simplifies the handling of solutions containing large concentrations of pigment, but is useful in studying dilute ones. Such solutions sometimes give very weak absorption bands, and the observer may be in doubt whether a band is present. If the band appears and disappears as the thickness of the solution is varied while the spectrum is under observation, more confidence is felt in the accuracy of the findings.

A convenient apparatus can be arranged for carrying out the examination by using a glass wedge from a Hellige colorimeter, as shown in the illustration. The wedge can be raised and lowered at will by a rack and pinion. If a permanent source of light is used and the slit in the spectroscope always opened to the same extent, the method can be made into a semi-quantitative one. This is done by preparing mixtures of sulphemoglobin and normal blood as described above and noting the lowest scale reading obtained when the band due to the abnormal pigment can be clearly distinguished.

The method described is convenient and delicate. The actual amounts which can be detected vary, of course, with the form of spectroscope used, and are different with different sources of light, but when blood is hemolized with saponin and diluted with an equal volume of distilled water as described, it is hardly possible

to miss significant amounts of pigment. It seems probable, however, that amounts of abnormal pigment which are of little or no clinical significance will sometimes be detected. It seems desirable to emphasize this possibility. Clinical cyanosis should probably not be explained as due to sulphemoglobinemia unless rather large amounts of the pigment (probably more than 1 gram per 100 cc.) are present, or unless all other possible explanations of the condition have been excluded by careful clinical observation and extensive laboratory study.

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SURVEY OF TRAINING SCHOOLS FOR LABORATORY TECHNICIANS*

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This survey represents a systematic attempt to ascertain, if possible, the present status of the so-called "training schools" for laboratory technicians and to assist in formulating an outline of a course of training which may be accepted as a basis for a minimal standard of such a course, exclusive of the courses in Medical Technology offered by universities and colleges of recognized standing. In all, 872 questionnaires were mailed, 376 to members of the American Society of Clinical Pathologists, 401 to superintendents of hospitals approved for interneship by the American Medical Association, of more than 150 beds, 85 to practicing clinical pathologists, not members of the Society and 10 others.

Information was obtained from 399 answers which is considered good and represents a fair cross section view of the training school situation. One hundred sixty-four (43 per cent) members answered, fifty-three (55 per cent) non-members answered and replies were received for 182 (45 per cent) hospitals. In all, 137 laboratories and schools which conduct a course of training for laboratory technicians answered the questions.

Fifty-four of the 164 members, not including members in hospitals, give courses for technicians, twenty-seven, twenty-four medical men and three laymen, of the fifty-three non-members give courses, and fifty-six of the 182 hospitals give courses, ten being conducted by members, forty-four by non-members and two by laymen. In other words of the 188 members heard from

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directly or indirectly, sixty-four (34 per cent) conduct courses for technicians. Of the 150 non-members who are physicians, sixty eight (45 per cent) give courses, while five (71 per cent) of seven laymen also give such courses. Thus, one-third of those replying to the questionnaire are conducting some kind of courses for training laboratory technicians.

Only one-third of the Society's membership and 45 per cent of the non-members responding, are offering a course of training for

TABLE 1
Types of Institutions

	NUMBER	PER CENT
Universities and Colleges:		
Organized course in Medical Schools leading to B.S.... 4	13	9.5
Organized postgraduate course leading to M.S... 1		
Organized course in College of Sciences leading to B.S.... 2		
Unorganized course leading to B.S.... 2		
Unorganized postgraduate course.. 4		
University hospital laboratories....	16	12.0
Hospital laboratories....	81	59.0
Public Health laboratories....	1	1.0
Private laboratories with hospital connection....	13	9.5
Private laboratories....	5	3.5
Private laboratories conducted by non-medical graduates....	3	2.0
Commercial schools....	5	3.5

technicians. Of the non-members, there are a number of recognized pathologists, several, members of the American Association of Pathologists and Bacteriologists or teachers of pathology in medical schools, while a few are known to be practicing physicians who are not trained in clinical pathology.

Geographically these courses are offered as follows: Middle west, 48 (35 per cent), East, 48 (35 per cent), South 27 (20 per cent) and West 14 (10 per cent).

Table 1 classifies the replies received from 137 institutions.

Doubtless, there are a number of other universities offering a

course which prepares the candidates as medical technicians but they have no announcement of such a special course nor have they responded to our inquiries. A large number of hospitals, not responding to this questionnaire, and the smaller institutions not included in this survey as well as pathologists who failed to respond are undoubtedly conducting a training course. The number is conservatively estimated to be at least 100.

TABLE 2

Size of Hospitals Offering Courses

NUMBER OF BEDS	NUMBER OF HOSPITALS	PER CENT	NUMBER OF BEDS	NUMBER OF HOSPITALS	PER CENT
32–99	10	8	300–399	22	17
100–150	13	10	400–499	15	11
151–199	18	14	500 and more	19	14
200–299	33	25			

TABLE 3

Length of Course

MONTHS	NUMBER COURSES	MONTHS	NUMBER COURSES
2	1	10	2
2	2	12	47
4	2	15	1
5	1	16	3
6	34	18	9
7	1	24	8
8	4	Post graduate	3
9	8	48 (B.S.)	7
		Not given	4

There are several teachers without hospital connections whereas a few pathologists have several hospitals.

Sixty-seven per cent of the hospitals have more than a 200-bed (table 2) capacity.

Twenty-seven per cent of the courses extend for a period of six months while 34 per cent last twelve months. Table 3 gives the summary of the length of courses offered.

High school education is considered sufficient prerequisite by a great majority (64 per cent) of pathologists. The exact requirements are given in table 4.

TABLE 4

PREREQUISITES

	NUMBER	PER CENT
No information or preference...............	9	6.5
High school................................	88	64.0
One year college...........................	8	5.5
Two years college..........................	11	8.0
College graduates.........................	11	8.0
Graduate nurse............................	10	8.0

TABLE 5

STUDENT CAPACITY

STUDENTS	SCHOOLS	STUDENTS		SCHOOLS
1	16	70*		1
2	29	Not given		5
3	17	Universities	6	1
4	17		40	2
5	8		50	2
6	12		65	1
8	6		100	1
9	1		Not given	6
10	1	Commercial schools	24	1
12	3		100	1
15	1		150	1
18	1		Not given	2
26	1			

* This institution may be considered a commercial school.

TABLE 6

INSTRUCTORS*

NUMBER INSTRUCTORS	NUMBER SCHOOLS	NUMBER INSTRUCTORS	NUMBER SCHOOLS
0	2	7	2
1	17	8	2
2	27	10	4
3	20	12	1
4	18	30	1
5	16	Faculty	11
6	7	No information	9

* Ratio of Instructors to Students 1:1.1, exclusive of Universities and commercial schools.

Formal lectures are given in the University and College Course, also, nominally, in the commercial schools. "Lectures" in the majority of the non-teaching hospitals probably mean "informal talks" such as given in any other laboratory. Sixty-nine replied that lectures are regularly given, 15 that some or few are given and 53 that no didactic work is offered.

Fifty-six per cent of the laboratories charge no fees (table 7) while a few charge what is considered an exorbitant fee, as much as or more than the commercial schools. The average fee for forty-three laboratories which charge for the course is $146.00. Including the seventy-seven laboratories which make no charges

TABLE 7

FEES

FEE	NUMBER OF SCHOOLS	FEE	NUMBER OF SCHOOLS
None	77	$125.00	2
$10.00 (Breakage)	2	150.00	6
15.00 (Breakage)	1	180.00	1
25.00	4	200.00	2
50.00 (Breakage)	3	250.00	1
50.00	5	300.00	2
75.00	2	360.00	1
100.00	7	400.00	4
120.00	1	600.00	1

and excluding twelve universities and colleges which charge regular tuition fees, the average fee for the course is about $54.00. Twelve charge the regular University fee. Three did not give information. Board and room was offered as a stipend by one laboratory; small stipends are given by two others.

OUTLINE OF THE SUGGESTED COURSES OF TRAINING FOR LABORATORY TECHNICIANS*

The training of laboratory technicians is, at present, being undertaken by the following institutions: (1) Universities and

*Approved by the Board of Registry of the American Society of Clinical Pathologists as a basis for further study.

colleges, (2) hospital laboratories, (3) Public Health laboratories, (4) private laboratories, and (5) commercial schools.

The first two types of institutions are considered properly adapted for the adequate training of clinical laboratory technicians and the third, for public health laboratorians. Private laboratories are not, as a rule, in a position to undertake such a program while the commercial schools, are, on the whole, considered unacceptable.

The ultimate aim of the Board is to get as many as possible of the universities and colleges to include in their regular curricula a course in Medical Technology either as a four-year course leading to a degree or on a two-year certificate plan. These courses must always be in affiliation with recognized general hospitals where the students shall obtain a part of the practical training as an "interne" or apprentice.

The apprenticeship plan of training in general hospital laboratories under proper supervision is recognized and recommended at the present time for the type of routine and special technicians which constitute the great majority of the present laboratory workers.

UNIVERSITIES AND COLLEGES

The following courses offered, at the present time, are:

(1) In connection with the College of Science, Literature and the Arts, and the Medical School and its hospitals.

(2) In connection with the College of Science, Literature and the Arts with or without the hospital affiliation.

(3) Postgraduate course (a) in various medical sciences, (b) in clinical pathology and medical technology.

(4) Unorganized, elective course.

The curricula recommended are as follows:

(1) A 4-year course leading to the B.S. in Medical Technology. The first two years to be essentially the same as the premedical requirements; the third year, didactic and laboratory studies in basic medical sciences and clinical microscopy and the fourth year, a rotating practical service in the clinical laboratory.

(2) A 2-year course leading to a certificate in Medical Tech-

nology.   The first year, didactic and laboratory hours, equivalent to regular college requirement in biology, chemistry, bacteriology and clinical microscopy.   The second year, a rotating practical service in the clinical laboratory.

Regular University regulations should govern the personnel, pre-requisites, method of instruction, student capacity, equipment, fees, advertising policy, et cetera.   Rotating practical service in the clinical laboratory of the affiliated hospital must constitute an integral part of this course.

Hospital laboratories (apprenticeship plan of instruction)

I. Personnel
    (A) Director, qualified clinical pathologist (or pathologist).
    (B) Medical staff.
    (C) Technical staff, supervising technician and associates, possessing the minimal qualifications defined by the Board of Registry of the American Society of Clinical Pathologists.
    (D) Ratio of instructors to students 1:2 or less, exception, teaching hospitals.

II. Pre-requisites (minimum)
    (A) High school graduation.
    (B) Credits of one-year college chemistry and biology or the equivalent.

III. Length of course (minimum)
    (A) Twelve months of apprenticeship training divided in 2 months (minimum 300 laboratory hours and 40 conference hours) each in the following departments:
        1. Urinalysis
        2. Clinical hematology.
        3. Clinical bacteriology and serology.
        4. Medical chemistry.
        5. Histopathologic technic and metabolism.
        6. Clinical microscopy.
        The above divisions are optional and may be modified.

(B) A longer course of 16, 18 or 24 months with corresponding increase in time spent in each department may be arranged.

(C) Twelve additional months of practical training, supplemented by a course of didactic study is required for specialization or advanced work on any of the above departments.

IV. Method of instruction

    (A) Practical training (abundant teaching material must be guaranteed).

        1. Personal instruction and demonstration.

        2. Practice period.

        3. Routine examination (under supervision).

    (B) Didactic hours:

        1. Required.

            a. Weekly conference.

            b. Quiz hour.

            c. Written examination.

        2. Optional.

            a. Medical lectures for nurses.

            b. Series of special lectures.

            c. Autopsies.

            d. Formal series of lectures on basis medical sciences.

V. Student capacity.

The average 100 bed general hospital employing two qualified technicians may enroll two students at a time. Hospitals of less than 100 beds are advised not to conduct a training school.

The Ratio of Instructors to students should be kept at 1:2 or less, in the average non-teaching hospital. Teaching and post graduate hospitals are excepted. Where there are more than six students enrolled at a time, a full time, supervising instructor-technician, besides the regular staff, may profitably be employed.

VI. Equipment, space and location.

Adequate equipment and space to meet the added requirements of training and practice of trainees.

VII. Fees.

A nominal fee (maximum $150.00) for a 12 months course, may be charged. Free instruction under ordinary circumstances, seems unwise. Charging of exorbitant fees on a commercial scale is not justified.

VIII. Advertising.

Commercial advertising should not be necessary. A short announcement in the medical and nursing periodicals may be permissible.

Public health laboratories

These laboratories are considered unadapted for the training of general clinical laboratory technicians. Special technicians and public health laboratorians may adequately be trained by these laboratories. The conditions under which the hospital laboratories should operate are applicable to the public health laboratories in their program of training.

Private laboratories

Private laboratories either directed by a clinical pathologist or operated by a non-medical director, are, as a rule, not adapted for the proper training of technicians. Those, maintaining such a course or school, though not equally obnoxious, must be regarded (with a few exceptions) in the same category as the commercial schools for laboratory technicians. Clinical laboratories of a small hospital (less than 100 bed capacity) are also considered, like the private laboratories, non-adapted for training of technicians. Of course, there are exceptions among these institutions.

Commercial schools

No attempt is being made, at this time, to regulate or standardize the existing curricula of these schools. As a rule, they are being operated by commercially minded promotors or direc-

tors, in the name of education and medical science. The objections against these schools are fundamental. They represent all that is contrary to the principles which the medical profession holds sacred and ethical. The commercial character of these institutions is clearly indicated by their advertising policy, the teaching facilities and personnel, the tuition they charge, the type of students they enroll, et cetera. They often advertise extensively in lay journals in exaggerated and misleading terms.

Teaching facilities are usually inadequate, more students being enrolled than can be properly accommodated either from the standpoint of the available space and equipment or from that of the teaching personnel. Teaching material is sorely inadequate both in number and variety of the specimens. Contact with the sick, representing various types of disease, is entirely lacking. The teaching force consists often of poorly trained technician with little or no teaching or technical experience. Educational prerequisites of the applicants are often disregarded in order to increase the enrollment. The tuition and incidental expenses are unusually high considering the kind of training they offer. A large majority of the "graduates" would be found lacking in qualifications essential in a properly trained technician.

Therefore, until these schools shall be directed by a clinical pathologist of high professional standing, are conducted on the sound educational basis with a staff of qualified teachers in sciences and a close affiliation with one or more general hospitals, and shall eliminate such objectionable features as extensive commercial advertising and propaganda and exorbitantly high rates of tuition, it would be useless to make any attempt to standardize the curricula of these institutions. It may safely be predicted that when the standardization of the courses of training for technicians offered by the recognized colleges and hospitals is accomplished, coupled with the awakening of the medical profession to the true character of these schools, the commercial institutions offering a course for laboratory technicians may cease to be the serious problem which they are now.

EDITORIAL

THE RELATION OF CLINICAL PATHOLOGISTS TO THE CANCER PROBLEM*

The American Society of Clinical Pathologists has had a short but honorable career, honorable because it has proved that its sole purpose is not to create offices to be filled but rather to carry on a profitable constructive program. Its coöperation with the American College of Surgeons in the past has been much appreciated. The nomenclature adopted by the Registry of bone sarcoma was arrived at after consultation between Fellows of the College and a committee of this organization.

To the individual members of this Society the College owes much on account of their close coöperation with our Department of Hospital Activities in its program of hospital standardization.

A clinical pathologist is an individual that it is a bit difficult to define, and the term bears a different significance in different parts of the country. It is indeed fortunate that the individual who carries on the laboratory work in the hospital has not allowed his activities to be circumscribed by any limited definition of his position. There is a tendency in some of the specialties of medical art and science to circumscribe rather too closely one's interests to the detriment of the specialty which is being practised. Fortunately this is not so with the clinical pathologist whose interests are all-embracing, or should be. We must remember that the dictionary still defines the pathologist as "one skilled in the science treating of diseases, their nature, causes, progress, results, et cetera." This definition sufficiently indicates the breadth of scope of the clinical pathologist's activities.

Within the very recent past specialization has grown up to a remarkable extent within the confines of what may be defined as pathology. We have bacteriologists, animal parasitologists, mycologists, immunologists, biochemists, epidemiologists, and so on almost *ad infinitum*, and each of these sciences now has enough sub-divisions to make the meticulous systematist tremble for the accuracy of his premises. If we might imitate the methods of the systematist, the pathologist now merits the dignity of an order —say, Knight Commander of the Laboratory. A member of this order in one direct line only might be indicated as follows:

> Family—*Zoologist*
> Tribe—*Parasitologist*
> Genus—*Helminthologist*
> Species—*Trematode immunologist*

and so this method might be elaborated to form a wide-spreading and comprehensive phylogenetic tree.

In my early days in the laboratory I did gross and microscopic pathologic anatomy and made some crude chemical and microscopic examinations of the body fluids. However, in the early days I did no Wassermann, Kolmer, or Kahn tests. I searched for no pathogenic treponema; rarely did a spinal fluid count; allergic and anaphylactic phenomena were not within my ken; pneumococci were not typed, vaccines were in their infancy, my laboratory was not cluttered up with colorimeters; basal metabolism was not a clinical test; the electrocardiograph was unknown; the endoscopist did not pester me with submicroscopic specimens; T. A. T. meant no more than the letters spell; pH meant nothing to me; the bi-polar theory of vital phenomena was still aborning in Cleveland; and so I could go on enumerating the advances in knowledge that have been made during my own medical career. Within the sphere of pathology I was a jack of all trades and worked for sixteen to eighteen hours a day but the majority of the activities of the present day clinical laboratory were unknown and unthought of. Still, I must admit that people were ill, some died, some recovered, and the accuracy of the diagnoses was not small.

You will see to what I am leading when I say that this is a day of minute specialization and that any man to know his subject well must have a wealth of experience in his own line. No longer can a country doctor with a Bunsen burner and a test tube for his laboratory equipment make the additions to knowledge that he once could. Nor can a man who has had a few months of laboratory training in a general hospital be an expert in all of the sciences whose fringes he must touch. What has all this to do with the clinical pathologist and his relation to cancer?

The American College of Surgeons through its Committee on the Treatment of Malignant Diseases has recently evolved a minimum standard for cancer clinics in general hospitals, which is as follows:

1. Organization. There shall be a definite organization of the service, and it shall include an executive officer and representatives of all the departments of the hospital which are concerned in the diagnosis and treatment of cancer. The services of a secretary and of a social service worker shall be available.

2. Conferences. As an essential feature of the service there shall be regular conferences or consultations at which the diagnosis and treatment of the individual cases are discussed by all members of the clinic who are concerned with the case.

3. Patients. Reference to the cancer clinic of all patients in whom the diagnosis or treatment of cancer is to be considered shall be either voluntary or obligatory in accordance with the vote of the medical staff or of the governing board of the hospital.

4. Equipment. In addition to the diagnostic and therapeutic surgical equipment which is required in every approved general hospital there shall be available an apparatus for x-ray therapy of an effectiveness which is generally agreed upon as adequate, and an amount of radium sufficient to insure effective treatment.

5. Records. In addition to the records which are required in every approved general hospital, there shall be additional records of: (*a*) The details of the history and of the examination for cancer in different regions of the body, such as are indicated on the form records which are recommended by the Committee on the Treatment of Malignant Diseases, American College of Surgeons. (*b*) The details of the treatment by radium or x-ray as indicated on the form records which are recommended by the Committee on the Treatment of Malignant Diseases, American College of Surgeons. (*c*) Periodic examinations at intervals for a period of at least five years following treatment.

6. Treatment. The treatment of cancer patients shall be entrusted to the members of the staff of the cancer clinic except in cases in which adequate treat-

ment in accordance with the collective recommendation of the staff of the cancer clinic can be procured otherwise.

This standard has just been published with an elaboration of its various clauses. I hope that you will all agree with me that it is fundamental. The underlying principles on which these recommendations are based may be stated in the form of four axioms:

1. The diagnosis and treatment of cancer is no longer a one man job.

2. The cancer patient merits the advantage of the best available medical knowledge.

3. The general practitioner who cannot be versed in all of the ancillary sciences should have made available to him the facilities and knowledge possessed by those versed in these sciences.

4. Immediate and complete records of all cases are necessary in order that trustworthy conclusions may be drawn as to the value of treatment administered.

It is difficult to forecast what the future organization of medical practice will be, and this is rather aside from our point but it seems obvious that the type of group practice which I am here defining is a necessary essential to medical progress. It takes nothing from the general practitioner but offers to him a service which at the present is available to relatively few and those chiefly in the larger medical centers.

In this standard emphasis is placed upon the coöperation of the surgeon, the radiologist and the clinical pathologist in arriving at a decision as to the diagnosis, prognosis and treatment of individual cases. This coöperation is essential for success and every medical organization must take cognizance of it.

The pathologist must equip himself specially to carry on this work in tumor pathology. There is rapidly coming into existence another specialty within a specialty. The expert tumor pathologist must know not only how to make a tissue diagnosis as to the cellular elements constituting the tumor under discussion but he should also know the natural course of such a tumor as it develops in different parts of the body. He must know its grade of malignancy. He must know its radiosensitivity. He must

be in a position to advise with the clinician and the radiologist as to the most effective method of treatment of the particular tumor under discussion. Such knowledge can only be gained by familiarity with a large group of patients.

Perhaps I can illustrate this well by saying that the preëminent position which Dr. James Ewing occupies among the tumor pathologists of this country is due in large part not to any of his academic attainments but rather to a careful personal scrutiny and analysis of large groups of tumor patients not only from the standpoint of the microscopic changes in the tissue but from the clinical and radiological standpoints. He has profitably associated himself with the clinicians and with the radiologists and has fortified himself with a knowledge of the subject of tumors from as many different angles as possible.

I do not wish to be held responsible for introducing more specialties and a greater confusion into the already almost indissolubly intricate maze of medical practice but I do wish to be considered among those who are working to bring the best available medical knowledge of the present day to bear on every individual cancer patient throughout the country. I recognize the value of cancer institutes and cancer hospitals, but I also recognize that economic conditions are such that the most practical means at present available of bringing the best of medicine to each of the quarter of a million of cancer patients throughout this country is by the utilization of already existing general hospitals. I have welcomed this opportunity of bringing this subject to your attention and I look for much assistance from you in putting this program into effect.

B. C. CROWELL.

SOCIETY NEWS AND NOTICES

Constitution and By-Laws on the American Society of Clinical Pathologists

CONSTITUTION

Article I—Name

This Organization shall be known as the American Society of Clinical Pathologists.

Article II—Objects

The objects of this society shall be: (a) To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease; (b) to stimulate original research in all branches of clinical laboratory work; (c) to establish from time to time standards for the performance of various laboratory examinations; (d) to elevate the scientific and professional status of those specializing in this branch of medicine; (e) to encourage a closer co-operation between the practitioner and the clinical pathologist.

Article III—Membership

Section 1. The membership of this society shall consist of (a) Fellows, (b) associate, (c) honorary, and (d) corresponding members.

Section 2. Fellows shall be graduates in medicine from a recognized medical school, who have specialized in clinical pathology for at least three years and who are devoting a major portion of their time to this field.

Section 3. Associate Members shall be graduates of recognized scientific institutions who have made such contributions to any of the sciences relating to clinical pathology and whose membership will so further the objects of the Society as to make them eligible for associate membership. Associate members shall pay the regular dues and have all the privileges of Fellows except those of voting and holding office.

Section 4. Honorary members shall have distinguished themselves by research or personal sacrifice in the cause of scientific medicine to warrant their recommendation for election by the Board of Censors. They shall have all the privileges of active members except that of voting and holding office. They shall be exempt from paying dues.

Section 5. Corresponding members shall be residents of foreign countries in good ethical standing who have distinguished themselves in any of the branches of clinical pathology. They shall be exempt from paying dues.

Article IV—Officers

SECTION 1.　The officers of this society shall consist of a President, President-Elect, Vice-President, Secretary-Treasurer, Executive Committee, Board of Censors, and Board of Registry of Technicians.

SECTION 2.　Officers shall be elected by a majority of the ballots cast at the annual executive business session.

SECTION 3.　The Executive Committee shall be composed of six Fellows of the Society, who shall each hold office for three years or until their successors are elected; two to be elected annually.

SECTION 4.　The Board of Censors shall be composed of six Fellows of the Society, who shall each hold office for three years or until their successors are elected; two to be elected annually.

SECTION 5.　The Board of Registry of Technicians shall be composed of six Fellows of the Society, who shall each hold office for three years or until their successors are elected; two to be elected annually.　The first Board shall consist of six Fellows, two of whom shall be elected for a term of one year, two for a term of two years, and two for a term of three years.　It shall elect its own Chairman from among the holdover members and Secretary-Treasurer.

SECTION 6.　Vacancies in the interim on the Executive Committee, Board of Censors, or the Board of Registry of Technicians shall be filled by appointment by the President.

Article V—Duties of Officers

SECTION 1.　The President shall preside at all meetings of the Society, be an ex-officio member of all committees and perform all other duties that devolve on him by custom and parliamentary usage.

SECTION 2.　In the absence of the President, the President-Elect, and in the absence of both, the Vice-President shall perform the duties of the President.

SECTION 3.　The Secretary-Treasurer shall keep a correct and permanent record of the meetings and the transactions of the Society.　He shall provide a copy of this record for all members in good standing, conduct the correspondence and perform such other duties as customarily pertain to the office of Secretary. He shall receive and keep funds of the Society and pay out of the same with consent of the Chairman of the Executive Committee.　He shall give bond satisfactory to the Executive Committee, the cost of which shall be borne by the Society.　He shall make a complete financial report at the annual meeting of the Society.　He shall serve a term of three years.

SECTION 4.　The Executive Committee shall audit the Treasurer's account annually or as often as they deem necessary.　The Chairman shall hold the Treasurer's bond.　The Executive Committee shal have general supervision of the financial affairs of the Society.

SECTION 5.　The Board of Censors shall investigate all applications for membership and submit their recommendations at the annual meeting of the

Society. They shall receive and consider all complaints concerning the conduct of members and present a report at the executive session, with their recommendations. Suspension or expulsion from membership in the Society shall be by three-fourths vote of those members present and voting at a regular executive session.

SECTION 6. The Board of Registry of Technicians shall conduct a Registry of Technicians, receive applications for such, pass on their qualifications and issue certificates to those meeting the requirements. They shall investigate schools for the training of technicians, registering those approved. They shall conduct a placement bureau for technicians.

Article VI—Amendments

This Constitution may be altered or amended by a vote of three-fourths of the Fellows voting at a regular meeting in executive session, provided said alteration or amendment had been submitted to the membership by publication or otherwise at least thirty days prior to the annual meeting.

BY-LAWS

Article I—Applications for Membership

Application for membership shall be made on a form authorized by the Society, signed by the applicant, recommended by two members, approved by the local Counselor and the Board of Censors. At least thirty days prior to the convention, the Secretary shall send a list of applicants to every member of the Society.

Article II—Qualification for Membership

SECTION 1. Fellows shall be graduates in medicine from a recognized medical school, who have specialized in clinical pathology for at least three years and who devote the major portion of their time to this branch of medicine. They must be members in good standing of their local and state society.

SECTION 2. Applicants for Fellowship, associate, and corresponding membership approved by the Board of Censors, shall be elected by a ballot of three-fourths of the Fellows voting at any regular meeting.

SECTION 3. Honorary members shall be recommended by the Board of Censors, and elected as in Section 2.

Article III—Dues

SECTION 1. All Fellows and Associate Members shall subscribe to this constitution at the time of their election to membership and shall pay an initiation fee of Twenty-Five Dollars ($25.00), payable with application for membership.

SECTION 2. The annual dues for Fellows and Associate Members shall be Ten Dollars ($10.00), payable December first for the following year, and if unpaid on January first the subscription for the Official Journal will lapse. Five

Dollars of the annual dues shall be used as subscription for the Official Journal. New members elected at the annual meeting shall pay dues for the current year of Five Dollars ($5.00) to cover the subscription of the entire volume of the Official Journal for that year.

SECTION 3. Members in arrears for dues for sixty days shall be notified thereof by the Secretary-Treasurer by means of a "return receipt" registered letter. Members in arrears for dues for ninety days shall be automatically dropped from the roll for non-payment of dues. Members may be reinstated upon payment of all arrears and current dues within one year without recommendation by the Board of Censors.

Article IV—Committees

SECTION 1. A Board of Counselors shall be appointed by the President. They shall represent such districts as may be determined. It shall be the duty of the counselors to act in the interest of the organization in their respective communities.

SECTION 2. A Nominating Committee of three shall be appointed by the President at the opening of the convention, whose duty it shall be to prepare a list of nominees for the various offices for balloting by the Society. Additional nominations may be made from the floor.

SECTION 3. The President shall appoint a Program Committee consisting of three Fellows, the Chairman of which shall be the Secretary of the Society, whose duty it shall be to arrange the scientific program for the annual meeting.

SECTION 4. The President shall appoint a Committee of Exhibits, of which the Secretary of the Society shall be a member whose duty it shall be to arrange for scientific and commercial exhibits at the annual meeting.

SECTION 5. The President shall appoint a Publication Committee.

SECTION 6. The President shall appoint a Research Committee.

SECTION 7. The Executive Committee shall appoint an Editor for the Official Journal of the Society for a term of three years. The Editor so selected together with the President of the Society and the Chairman of the Executive Committee of the same shall appoint an Advisory Editorial Board for a period of three years. The duties of this Advisory Editorial Board shall be to foster and supervise all official publications of the Society. The three year term of office of the first Board so appointed shall expire on January first 1934. Nothing in this Section shall prevent continuing a Publication Committee which has not yet discharged its duties.

Articles V—Quorum

Twenty-five Fellows shall constitute a quorum.

Article VI—Meeting Place

SECTION 1. The time and place of the annual meeting and other meetings of the Society shall be determined by the Executive Committee, notice of which shall be mailed to every member at least thirty days prior to such meetings.

Article VII—Elections

SECTION 1. The Society shall elect annually by ballot at an executive session held on the last day of the annual meeting the following officers: President-Elect, Vice-President, Secretary-Treasurer, two Fellows to fill vacancies on the Executive Committee, two Fellows to fill vacancies on the Board of Censors, and two Fellows to fill vacancies on the Board of Registry of Technicians.

SECTION 2. The President-Elect and newly-elected officers shall be inducted into office at the conclusion of the meeting.

Article VIII—Code of Ethics

SECTION 1. The Code of Ethics of this Society shall be the same as that of the American Medical Association.

SECTION 2. It shall be deemed unethical for members to publish objectionable laboratory advertisements in any form whatsoever. The Board of Censors to act as judges in the matter, the members having privilege of appeal to the Society at a regular executive session.

SECTION 3. It shall be considered unethical for a member to lend his name for publication in any laboratory advertisement or announcement, which violates the Code of Ethics. The borrowing of names of other physicians, scientists or laymen, on the basis of an occasional service or consultation, for purposes of advertising or to sanction the work of a laboratory is misleading and unethical.

SECTION 4. Any system of secretly dividing or rebating fees for laboratory services shall be considered unethical.

Article IX—Standing Rules

SECTION 1. The Chairman, at all regular annual meetings, shall first call the members assembled to order in executive session for the purpose of transacting such business and appointing such committees as are herein required, together with the making of other arrangements consistent with conducting the annual meeting.

SECTION 2. Scientific papers limited to twenty minutes for members, thirty minutes for guests, longer only on consent of the majority.

SECTION 3. Opening discussion limited to ten minutes, all succeeding discussions five minutes except by consent of the majority.

SECTION 4. Members desiring to speak twice must obtain consent.

SECTION 5. Non-members can be given the privilege of the floor only by consent.

SECTION 6. A paper read before this Society becomes the property of the Society, to be published in the Official Journal provided it meets the approval of the Advisory Editorial Board, except that the privilege for prior publication may be granted by the Editor.

SECTION 7. Order of Business for Executive Session:

1. Call to order.
2. Reading of Minutes.
3. Unfinished business.
4. Reports of committees.
5. Election of members.
6. New business.
7. Nominations.
8. Election of officers.
9. Induction of officers.
10. Adjournment.

Article X—Parliamentary Procedure

All parliamentary proceedings at the meetings of this Society shall be governed by Roberts' Rules of Order, except where otherwise provided.

Article XI—Amendments

Amendments of these by-laws must be submitted in writing at the opening of the annual meeting and may be voted upon at the executive business session.

The attention of the Society is called to the following changes in the By-Laws: Article III, Section 2 relates to the subscription to the JOURNAL and specifies that $5.00 of the annual dues shall be used for such subscription for each member. The Section also covers the cost of the JOURNAL to new members.

Section 3 of the same Article, refers to the change in the method of notifying members in arrears for dues.

Article IV, Section 3 provides that the Secretary shall be Chairman of the Program Committee. Section 4 of the same Article provides that the Secretary shall be Chairman of the Committee on Exhibits. Section 7 is an addition providing for the Editor and Editorial Board of the official JOURNAL of the Society.

Article IX, Section 6 has been slightly changed to provide for the approval of papers by the Advisory Editorial Board before their publication in the JOURNAL.

The Secretary announces the following Committees appointed by the President:

Research Committee

ALVIN G. FOORD, *Chairman*
ERNEST SCOTT
M. PINSON NEAL

Necrology Committee

HARRIET J. LAWRENCE, *Chairman*
W. G. GAMBLE
HERBERT R. MILLS

Program Committee

A. S. GIORDANO, *Chairman*
ALBERT H. BRADAN
C. E. RODERICK
HERMAN SPITZ

Public Relations Committee

B. W. RHAMY, *Chairman*
F. B. JOHNSON
G. S. GRAHAM
J. J. MOORE

Vaccine Therapy Committee

R. A. KEILTY, *Chairman*
JOHN A. KOLMER
JOHN EIMAN
A. B. HUNTER
B. E. STOUT
J. H. BLACK
RUTH GILBERT

The Chairman of the Program Committee, Dr. A. S. Giordano, is ready to receive titles from those desiring to present papers at the next annual meeting which will be held on May 6–9, 1932 at New Orleans, Louisiana.

The Research Committee after due deliberation and consultation with officers in the Society has decided on the following subjects for research activities of the Society for the coming year. Some of the problems or subjects may not appeal to all the members, but at the same time they should be of interest to most of the members engaged in hospital diagnostic work. Hematologic material is stressed at this time because of the simplicity of recording and compiling. In later years more involved studies can be taken up.

1. The hematologic registry will be continued and a summary of the cases will be presented at the next meeting of the Society. Reports are desired on acute leukemia, acute mononucleosis, agranulocytic angina, and blood dyscrasis following treatment by the arsphenamines, and cases showing a blood picture resembling pernicious anemia, but in which a definite etiology is proved. All the records and slides of these cases will be on file and when sufficient numbers are on hand they will be loaned to the various members of the Society on request. Cases should have a good history, complete blood counts, and blood smears, perferably at intervals, and slides of bone marrow, lymph nodes, spleen or

other organs of interest. Questionnaires for cases of acute leukemia and agranulocytic angina will be sent from the Secretary's office; other cases can be reported without a questionnaire. Please ferret out recent cases and send them as soon as possible to the Chairman of the Committee.

2. It is also recommended that each member who meets with a case of hemophilia, thoroughly study the case by appropriate laboratory tests including clotting time of venous blood and prothrombin time, simultaneously with a normal control, and try the effect of subcutaneous injection of ovarian extract as reported by Carroll Birch (Proc. Soc. Exp. Biol. & Med., April, 1931, page 752, and Jour. Am. Med. Assn., July 25, 1931). Birch's results warrant a widespread trial of the method, and only by the pooling of the results of a large number of cases can a reasonable impression of the efficacy of the treatment be determined.

3. Reports are also desired of the blood findings in cases of broad tapeworm infestation. Ordway's questionnaire to a limited number revealed practically no cases of blood changes. Please give a short synopsis of case with laboratory record and blood smears if any have been kept.

4. Because of the paucity of follow-up records of cases of splenectomy for thrombocytopenic purpura the Committee recommends reporting all cases at this time. By doing so information of several hundred cases would be available for study by any one desiring to use the loan collection. Please send history, laboratory records and blood smears and sections of the spleen if available, but if no slides are on hand do not neglect to send in the case record without them. Recent or old proved cases are satisfactory.

5. The Committee recommends keeping records of the results of the Friedman urine test for pregnancy, using the rabbit as recommended by Reinhart and Scott (Am. Jour. Clin. Path., February, 1931). Just before the meeting in New Orleans the data will be collected. Especially should tests be done in cases of chorioepithelioma, suspected abortion or abdominal pregnancy, most of which occur too rarely in any one hospital for collection of any large series.

6. Finally, the Committee recommends culturing the blood in cases of chronic rheumatoid arthritis according to the method used by Cecil, et al., (Jour. Exp. Med. Nov. 1, 1929). Cecil's findings of more than 60 per cent positive *Streptococcus hemolyticus* cultures (Jour. Am. Med. Sciences, Jan. 1931, page 12) have been corroborated by one of our members, Gray (Jour. Med. Soc. of New Jersey, Jan. 1931, page 38). No doubt many of our men have been asked to try Cecil's method. By pooling results it can be established whether or not the method is satisfactory for general use. Possibly this work will link in well with the activities of the Vaccine Therapy Committee.

It is hoped that all the members will feel that the cases reported to the registry can be used in other publications. The registry will merely file them and keep them on record for the benefit of the Society members who wish to go over the material. No one member sees enough of the conditions mentioned above to know the whole story of the diseases. As evidence of this it is interesting to note that some of the cases reported as agranulocytic angina two years ago were really acute leukemias. Many members have in their files a case or two or more of the above conditions, but not enough to furnish material for a paper. These cases are practically buried but can be used if properly collected. Start sending material as soon as possible and do not wait until just before the meeting, for the Committee must have time to go over the material. A short resumé of a case is satisfactory but nothing surpasses a well-written one or two page case report. Please see that all pertinent blood examinations are made on all cases, repeatedly if possible. Remember the registry is yours and will be only as interesting as you make it.

Please send material to the Chairman of the Research Committee.

A. G. FOORD, *Chairman,*
Pasadena Hospital,
Pasadena, California.

A communication has been received from the Italian journal, Diagnostica e Tecnica di Laboratorio, to the effect that members

of the American Society of Clinical Pathology are entitled to receive the journal at the reduced price of 75 lire (about $4.00). The journal is published at Naples, Piazza S. Domenico Meggiore, 9.

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